REFLECTIONS
ON
RENAL FUNCTION

By

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PREFACE

For several years I have given a few lectures upon the physiology of the kidney to a small class reading physiology for Part II of the Natural Sciences Tripos in Cambridge, and have often been urged to have the lectures published. There is no convenient book to bridge the gap between the chapters in general text-books and larger treatises such as that of Homer Smith, and so I have ventured to publish, not the lectures exactly as they were given, but a small and rather informal book written in the spirit of the lectures from the same notes. This is not intended to be a comprehensive review of the literature of renal physiology, but aims at presenting in moderate compass a picture, sometimes an impressionistic one, of the working of the kidneys and of its adjustment to meet the changing needs of the body.

Renal physiology is controversial, and perhaps ought to be more so There is a temptation, especially when teaching, to seek clarity by a too well organized presentation which gives the impression of a greater understanding than we really have. I have tried to steer a middle course, and to present a clear picture without concealing its incompleteness and its lack of finality. In so far as fundamentals have been stressed, the approach is elementary; but a certain amount of new interpretation and integration, as well as of emphasis on the unsolved problems of the processes which underly renal function at the cellular level, makes it in some ways advanced at the same time. I cannot claim in so short an account to have been completely fair to all parties in controversy, but I hope that important points at issue have not been ignored, and ask the indulgence of any whose views have been too lightly passed over.

Friends suggested that a book without references would be refreshing, but the reader needs some guidance to sources. A few important secondary sources have therefore been mentioned in an introductory note, and primary sources which they quote have not been included in the text unless they are of especial interest; but references to some more recent work have been included. The *references quoted during a lecture cannot* be consulted until a later occasion, and it is hoped that the picture outlined in this book can be grasped as a whole without consulting the bibliography for the filling in of detail.

It is a pleasure to acknowledge the constant encouragement of Prof. R. A. McCance, C.B.E., F.R.S , and of other members of the Department of Experimental Medicine. My thinking about the kidneys has grown up in the atmosphere of this Department, and without its stimulus the book would never have been written. I also wish to thank the publishers for the friendly co-operation which made the path to print so smooth.

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I. INTRODUCTION

A NOTE ON SOURCES

The literature of renal physiology is extensive and widely dispersed through a large number of journals primarily devoted to physiology, zoology, anatomy, pathology, clinical medicine and paediatrics. Homer Smith's treatise [85] is an invaluable guide to the contents of more than 2,000 original contributions published up to 1951. The shorter and more closely knit account of renal physiology in the first edition (1937) is still worth reading. [83] Some special aspects are treated more fully and make pleasanter reading in the "Lectures on the Kidney" published in 1943. [84] Cushny's classical monograph [15] reviews some earlier material which is still interesting. Another treatise by Wolf, [113] with over 1,000 references, surveys the field in somewhat different fashion. This is a stimulating book because it is critical, provocative, and iconoclastic; and although the invention of a new and unfamiliar jargon makes it also at times irritating, some parts of the book are extremely illuminating. During 1950 the American Journal of Medicine published a series of "Seminars on Renal Physiology" which provide compact reviews of particular aspects of the subject; some of these will be quoted individually in the text

FUNCTIONS OF THE KIDNEY

The principal functions of the kidney are conveniently summarised under three headings·

1. *Excretion* The kidneys excrete, in aqueous solution in the urine, end-products of metabolism such as urea, uric acid and creatinine, as well as soluble foreign substances which have been

introduced into the body, or products of their detoxication. They also excrete the surplus of normal constituents of the body such as sodium chloride and water which have been absorbed in excess of requirements. The urine contains small amounts of other soluble constituents of the body, such as amino acids, especially glycine, serine, threonine, lysine and histidine, but these are thought of as leaking out or escaping rather than as being excreted In so far as only the surplus of many solutes is excreted, and the rest is retained, the kidneys perform a second major function:

2. *The conservation* of the soluble constituents of the body, such as glucose, inorganic salts, amino acids and other metabolites, and of the water which holds all of these in solution By striking a balance between the conservation and the excretion of water and of the substances dissolved in it, the kidneys perform a third most important function

3. *Regulation* of the chemical structure of the body fluids. This includes regulation not only of the *total concentration* or *osmotic pressure* of these fluids, and of their detailed *chemical composition*, including their pH, but also of their *volume*. Provided that intake in the diet is sufficient, *the kidneys play a major role in regulating* the concentrations of water, sodium, potassium, chloride, phosphate, calcium, glucose and urea in the plasma, and they take a large part in the long-term regulation of acid-base balance. [illegible] which they regulate the amount of water in the

[illegible]

the cells in the body, not merely of those within the kidney. So long as metabolism continues normally in the tissues, the sodium of the body is mostly extracellular, whilst most of the potassium is in the intracellular fluids. This distribution is maintained in defiance of a concentration gradient across every cell membrane by energy derived from metabolic processes in the cells in a manner which still remains to be elucidated. But while

this unequal distribution of ions persists, the cell membranes behave *as if* they were semipermeable, and the volume of the cells is determined by the osmotic pressure of the fluids outside them, most of which is contributed by sodium salts. Hence if the amount of sodium in the body remains constant, the kidneys can regulate the osmotic pressure of the extracellular fluids by adjusting the excretion of water, and in doing this they can control the volume of the cells. On the other hand, so long as the osmotic pressure of the extracellular fluids is kept constant by controlling the excretion of water, the total volume of the extracellular fluids (plasma, lymph and the interstitial fluids) can be increased or decreased by retaining or excreting sodium. The kidneys can therefore regulate the volume of fluid inside the cells by the *controlled excretion of water, and the volume of fluid outside* the cells by the controlled excretion of sodium.

The kidneys may be regarded as organs which help to stabilize the internal environment of the body and to keep it chemically distinct from its surroundings The elimination of waste products is but one aspect of this much larger task. Other functions, such as the long-term regulation of blood pressure, have been postulated, but will not be discussed here. The two main questions to *be considered are, first, how the kidneys do what they do, and*, secondly, how they know what to do, or more properly, how they are directed to do it. This second question is concerned with the kidney as one part of the body in relation to other parts, and is for that reason more complex. The simpler question of the mechanism of the kidney itself will be taken up first.

STRUCTURE AS A CLUE TO FUNCTION

Certain structural features of the kidney which bear upon its mechanism will now be summarised. Each human kidney consists of about a million more or less similar units, called "nephrons", arranged in parallel. Each nephron commences in a glomerulus, which leads in turn to a proximal convoluted tubule,

a loop of Henle, and a distal convoluted tubule. The loop of Henle includes a thin descending limb which varies in length from one nephron to another, and a thicker ascending limb lined by a columnar epithelium continuous with that of the distal convoluted tubule. The proximal and distal convoluted tubules lie near the corresponding glomerulus in the cortex of the kidney; the descending limb of the loop of Henle runs down radially into the medulla, to turn back upon itself at a hair-pin bend. The distal convoluted tubules end in collecting tubules which descend again into the medulla but do not return; they drain instead into the renal pelvis.

Although they are similar in pattern the nephrons are not all alike. They differ most strikingly in their vascular arrangements, which depend upon the position of their glomeruli within the kidney. Nephrons with glomeruli lying in the outer two-thirds of the cortex — called *cortical nephrons* — are the more numerous They were developed embryologically later than the nephrons more deeply placed in the kidney, and their loops of Henle do not descend so far into the medulla; the thin segments are short, and may be altogether absent. The blood leaving a cortical glomerulus by its efferent arteriole is collected into an intertubu-

medullary nephrons. These have larger glomeruli than the cortical nephrons, their loops of Henle plunge more deeply into the medulla, and have longer thin segments. The efferent arterioles of juxta-medullary glomeruli differ in two ways from those of the cortical glomeruli. They are wider, instead of narrower, than the afferent arterioles, and they do not lead into intertubular capillaries, but drain instead into wide, straight, thin-walled venous channels called vasa recta which run in bundles parallel to the loops of Henle in the medulla. Like the loops of Henle the vasa recta descend for a variable distance into the medulla

and then turn abruptly back towards the cortex, where they end in veins. Both sets of tubes run radially, the bundles of vasa recta are embedded in the mass of loops of Henle, and individual loops are also interposed between the vasa recta within the bundles. The vasa recta are wider than capillaries, but their walls are made of the same kind of endothelium, so that this whole arrangement appears well suited to promote equilibrium by diffusion between the fluid in the loops and the venous blood in the vasa recta

The convoluted tubules have a "secretory" type of epithelium. The cells are columnar with basal striations formed by a parallel packing of mitochondria at right angles to the basement membrane The proximal convoluted tubules show in addition a brush border at the luminal poles of the cells. The cells lining the thick ascending limb of the loop of Henle resemble those of the distal convoluted tubule. Those of the thin limb are not much thicker than a capillary endothelium, but their nuclei may be seen bulging out into the lumen. Sjostrand & Rhodin (1953) have published illustrations of structural details revealed by the electron microscope in epithelial cells of the kidneys of mice [22] Histological evidence therefore suggests that the cells lining the tubules might secrete like those of other glands; the glomeruli, on the other hand, look like ultrafilters, and their simpler function will be considered before the more complex processes of tubular secretion.

II. GLOMERULAR FILTRATION AND TUBULAR REABSORPTION

GLOMERULAR FILTRATION

Over a century ago the structure of the glomeruli suggested to Bowman that they might excrete the water and salts of the urine. Bowman's capsule encloses a tuft of capillary loops arranged in parallel between a wider afferent and a narrower efferent arteriole. The afferent vessel arises from an interlobular artery, which has arisen fairly directly from the renal artery. The renal arteries themselves are short and wide and arise from the abdominal aorta. By this arrangement, blood at a pressure of one-half to two-thirds of that in the aorta is exposed in a layer a few microns in thickness over a thin membrane which consists of a single layer of endothelial cells supported on a basement membrane. This thin membrane in man has about the same total area as the external surface of the body. The glomerulus thus presents the appearance of an ultrafilter, and the fact that no urine is formed unless the head of pressure available for ultrafiltration exceeds the colloid osmotic pressure of the plasma proteins suggests that it functions as such. More direct evidence has been obtained in Amphibia, whose glomeruli resemble those of mammals in structure but are nearer to the surface of the kidney; Richards and his colleagues collected the glomerular fluid in tiny quartz pipettes pushed into the capsular space, and analysed it chemically.[75] No more than a trace of protein was present, and although the quantities of fluid obtained were too small to be analysed with great accuracy, *the glomerular fluid had the composition of an ultrafiltrate of plasma within the limits of the methods employed.*

In particular, it contained glucose when the urine in the bladder did not, and about as much chloride and urea as the plasma when the urine contained less chloride and more urea. The general similarity to plasma suggests filtration of protein-free fluid as a whole, rather than transpiration by diffusion, which might be expected to alter the proportions of the various solutes according to their diffusion coefficients (inversely proportional to the square root of the molecular weight).

The glomeruli of mammalian kidneys are less accessible to puncture than those of Amphibia, but Walker, Bott, Oliver & MacDowell[102] managed to withdraw fluid from different parts of the nephron in rats and guineapigs, and found that the fluid obtained when the proximal convoluted tubule was punctured nearest to the glomerulus resembled an ultra-filtrate of plasma most closely. If the mammalian kidney is chilled or poisoned with cyanide, either of which might be expected to stop the secretory activity of the epithelial cells, the urine comes to resemble an ultrafiltrate of plasma in chemical make-up. It is reasonable to conclude that the glomeruli in mammalian kidneys, like the similar ones in the kidneys of Amphibia, produce an ultrafiltrate of the blood plasma by a physical process which is nonselective for substances of low molecular weight. No work need be done by the glomerular cells in this process. Energy imparted to the blood by the beating heart and stored in the distended elastic walls of the aorta and renal arteries supplies the hydrostatic pressure required to separate the proteins from the other constituents of the plasma. Although this process is strictly one of ultrafiltration, common usage, which will now be followed, refers to it as "glomerular filtration" and to the ultrafiltrate produced as the "glomerular filtrate".

GLOMERULAR FILTRATION IN MAMMALS

Although the capsular fluid of the mammalian kidney cannot be collected and analysed, the rate at which it is formed can be

estimated in intact animals including man without drastic surgical procedures Apart from its practical value in the assessment of renal function for clinical purposes, a knowledge of the rate at which the glomerular filtrate is formed is important theoretically because it enables the part played by the tubules in the elaboration of the urine to be discovered. If the rate at which a substance appears in the glomerular filtrate exceeds its rate of excretion in the urine, then the cells which line the tubules must be reabsorbing it; on the other hand a substance which appears in the urine more rapidly than it is filtered by the glomeruli must be actively excreted by the tubular epithelium Most workers are content to assume that the composition of the glomerular filtrate may be determined by analysing plasma or serum. Non-electrolytes are taken to have the same concentration in the filtrate as in arterial plasma, but the concentrations of electrolytes in the plasma are [illegible]

and 0 90 for potassium, when there are 6 grams of protein in each 100 ml. of plasma. Because an appreciable fraction of the volume of the plasma is occupied by the proteins, the concentrations of solutes in the watery phase of the plasma are about 6% greater than their concentrations in whole plasma. Hence in order to obtain the actual concentrations of solutes in the glomerular filtrate, it would be necessary to multiply all concentrations determined by the analysis of whole plasma by a factor of about 1 06 as well as by any Donnan factor which might be required. This is a correction which is hardly ever made for a reason which will shortly become apparent

EXCRETION RATES AND CLEARANCES

The rate which a substance X is excreted in the urine is $U_x \cdot V$, where U_x is the concentration of X in the urine, and V is the rate at which urine is formed. V is most commonly expressed in ml.

per minute, and is known as the "minute volume". If U_x is expressed in mg. per ml., $U_x \cdot V$ will be the rate of excretion of X in mg per minute. Unless U_x and V are both constant throughout the period during which urine is collected for analysis, $U_x V$ will be an average and will not necessarily represent accurately the rate of excretion of X at any actual moment during that period. The renal pelvis and ureter constitute a "dead space" interposed between the nephrons and the bladder. Consequently there is a delay of several minutes before a substance which reaches the kidneys in the arterial blood appears in the urine in the bladder. A still longer delay, which may be as much as 20 to 30 minutes, is required for the concentration of a substance in the bladder urine to become constant after a steady concentration has been established in the arterial blood. Because of the delays introduced by the renal dead space, experiments performed while the concentration of a test substance in the plasma is rising or falling may give misleading results. Measurements are best made after a steady state has been reached in which V is constant and the composition of the urine and of the plasma are no longer changing. This is an ideal state of affairs which may be approached more or less closely in actual experimental work, but which is assumed in the subsequent discussion. In such a steady state, the average rate of excretion $U_x \cdot V$ becomes identical with the actual rate of excretion throughout the experimental period.

The rate of excretion $U_x V$ might be expected to depend, among other factors, upon the concentration P_x of X in the plasma. For many substances, at least if P_x is within certain limits, $U_x V$ has been found to be proportional to P_x. It is therefore reasonable, when comparing the excretion of these substances by the kidneys of different individuals or on different occasions, to "correct" the rate of excretion for the concentration presented to the kidneys in the plasma, and to calculate the ratio $\frac{U_x V}{P_x}$. This gives the rate of excretion of X per unit of

its concentration in the plasma. Since it is a rate of excretion divided by a concentration, it is not merely a numerical ratio, but has the dimensions of a volume per unit time. If $U_x . V$ is in mg. per minute, and P_x is in mg. per ml., then $U_x . V/P_x$ is in ml. of plasma per minute. It is in fact the smallest volume of plasma from which the kidneys could obtain the amount of X which they excrete each minute. If every minute the kidneys cleared the substance out of this volume of plasma completely, and transferred it to the urine, the observed rate of excretion would be accounted for; and so $U_x . V/P_x$ has come to be known as the "Plasma clearance of X". This must not be taken to imply that the kidneys do in fact clear $U_x . V/P_x$ ml. of plasma completely; in general they clear a larger volume incompletely. The "Clearance" is to this extent an abstraction, not a real but a virtual volume of plasma, and a property of the substance X rather than of the rate at which plasma circulates through the kidneys. When many substances are being excreted simultaneously, each has its own clearance, and these may differ widely from each other, but there is only one rate of circulation of plasma through the kidneys.

Clearances are frequently determined, and may be employed for any of the following purposes:—

1) To compare the excretion of a substance by the kidneys under different conditions or on different occasions; for instance during exercise or fever, or during the course of a progressive disease.
2) To compare the excretion of a given substance by the kidneys of different individuals or species.
3) To compare the renal excretion of different substances simultaneously or under similar conditions.
4) With suitable choice of substances, to determine the rate at which the glomerular filtrate is formed, or the rate at which blood circulates through the kidneys.

THE DETERMINATION OF GLOMERULAR FILTRATION RATES

Any substance which has the same concentration in the glomerular filtrate as in the plasma, and which is neither added to the urine nor removed from it by the tubular epithelium, may be employed to determine the glomerular filtration rate Such a substance can only enter the nephrons by filtration through the glomerular membrane, and its only way out is by conduction along the tubules into the urine. It must therefore be excreted at the same rate as it is filtered. The rate of excretion is, as before, $U_x . V$. The rate of filtration is $F. P_x$, where F is the volume of plasma filtered each minute Since these two rates are equal,

$$F . P_x = U_x . V . \quad \ldots \quad \ldots . \quad \ldots \quad (1)$$

$$\text{or } F = \frac{U_x . V}{P_x} \quad \ldots \quad \ldots . \quad . \quad . \quad \ldots . . \quad (2)$$

Hence the plasma clearance of a substance which is excreted only by glomerular filtration with no participation by the tubules is equal to the rate at which plasma is filtered This is by common consent referred to as the glomerular filtration rate, abbreviated GFR. It is important to notice that it is not the same as the rate at which the glomerular filtrate is formed. This would be obtained by dividing the rate of excretion, $U_x . V$, by the concentration of X in the filtrate, which if X is a non-electrolyte is the same as its concentration in the plasma water and about 6% greater than P_x. P_x may be regarded as the average concentration of X in a plasma which consists of —

i) 94% of water which contains X at a concentration P_x /0 94 and is filtered, and
ii) 6% of plasma protein which contains no X and is not filtered.

The actual rate at which the glomerular filtrate is formed is therefore about 94% of the glomerular filtration rate determined by equation (2), which defines the least volume of plasma from

its concentration in the plasma Since it is a rate of excretion divided by a concentration, it is not merely a numerical ratio, but has the dimensions of a volume per unit time. If $U_x \cdot V$ is in mg. per minute, and P_x is in mg. per ml., then $U_x \cdot V/P_x$ is in ml. of plasma per minute. It is in fact the smallest volume of plasma from which the kidneys could obtain the amount of X which they excrete each minute. If every minute the kidneys cleared the substance out of this volume of plasma completely, and transferred it to the urine, the observed rate of excretion would be accounted for; and so $U_x \cdot V/P_x$ has come to be known as the "Plasma clearance of X". This must not be taken to imply that the kidneys do in fact clear $U_x \cdot V/P_x$ ml. of plasma completely; in general they clear a larger volume incompletely. The "Clearance" is to this extent an abstraction, not a real but a virtual volume of plasma, and a property of the substance X rather than of the rate at which plasma circulates through the kidneys. When many substances are being excreted simultaneously, each has its own clearance, and these may differ widely from each other, but there is only one rate of circulation of plasma through the kidneys.

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3) To compare the renal excretion of different substances simultaneously or under similar conditions.
4) With suitable choice of substances, to determine the rate at which the glomerular filtrate is formed, or the rate at which blood circulates through the kidneys.

3) It should not be metabolized, for a substance which is metabolized cannot be presumed unable to enter cells, and might cross the tubular epithelium in either direction. In particular, it must not be synthesized, destroyed or stored within the kidney.
4) It should not be excreted by animals whose kidneys lack glomeruli This criterion gives a guarantee against tubular excretion only in so far as it is safe to assume similar behaviour in different species, and gives no guarantee at all against tubular reabsorption. Glucose, which is not excreted by aglomerular kidneys, is efficiently reabsorbed by mammalian tubules.
5) It must not combine with plasma proteins, and must be freely filtrable *in vitro* through membranes of comparable permeability to the glomerular capillaries; for the clearance of a substance which is not completely filtrable must underestimate the glomerular filtration rate.
6) The clearance of a substance suitable for measuring glomerular filtration rates should remain constant despite large variations in the minute volume of the urine, and of its concentrations in the plasma and in the urine. This would exclude a substance whose administration affected the filtration rate even if its clearance measured that rate perfectly, and it would seem to require the glomerular filtration rate to be constant as a condition of its being measurable. Any substance which was known to influence the glomerular filtration rate would be excluded by (1), but the filtration rate might vary for other reasons than effects of the test

which the protein-free filtrate formed in each minute could be derived. The rate at which any non-electrolyte enters the nephrons by filtration is its concentration *in the filtrate* multiplied by the rate at which the *filtrate* is formed. But since the concentration in the plasma underestimates the concentration in the filtrate as much as the glomerular filtration rate of equation (2) overestimates the rate of formation of filtrate, the rate at which any substance enters the nephrons by glomerular filtration is correctly given by the product of its concentration *in the plasma* and the rate at which *plasma* is converted to glomerular filtrate, that is, by the product of the plasma concentration and the conventional glomerular filtration rate. Since the correction of the conventional *GFR* to give the true rate of formation of filtrate would *cancel out the correction of the plasma concentration to give the* actual concentration in the filtrate, both corrections may be ignored. It is indeed important not to correct for the smaller water content of the plasma compared with the filtrate, for if this correction is applied and the conventional glomerular filtration rate is taken to be the rate of formation of filtrate, the amounts of the various solutes entering the nephron by filtration will be over-estimated.

The important question next arises whether any substances exist which are excreted solely by glomerular filtration and can be employed to determine glomerular filtration rates. The search for suitable substances has been governed by a number of criteria which have been developed and discussed in detail by Homer Smith, [85] and which need only be summarised with few comments.

1) A substance suitable for the determination of glomerular filtration rates must be non-toxic and physiologically inert. In particular, it must not be known to influence renal function.
2) It must be capable of accurate chemical estimation in blood and urine.

3) It should not be metabolized, for a substance which is metabolized *cannot* be presumed unable to enter cells, and might cross the tubular epithelium in either direction. In particular, it must not be synthesized, destroyed or stored within the kidney.
4) It should not be excreted by animals whose kidneys lack glomeruli. This criterion gives a guarantee against tubular excretion only in so far as it is safe to assume similar behaviour in different species, and gives no guarantee at all against tubular reabsorption. Glucose, which is not excreted by aglomerular kidneys, is efficiently reabsorbed by mammalian tubules.
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rather than with its weight. The glomerular filtration rate per unit of body surface turns out to be about the same at all ages greater than two years. This method of expressing the result is however less satisfactory for small babies, because it leads to the *conclusion that their glomerular filtration rates are lower than* those of adults until they reach about two years of age; whereas the kidneys of infants are adequate for their smaller needs, as judged by other standards. Thus they excrete urea at a sufficient rate to keep the blood urea of infants no higher than that of adults. The question therefore arises whether the kidneys of infants are really inferior to those of adults in respect of glomerular filtration, or whether they have merely been made to appear so by the unsuitable choice of a basis of comparison. McCance and Widdowson [56] have recently suggested that since water forms a larger percentage of the body weight of infants than of adults, the greater proportion of extracellular fluid in their bodies might be associated with a different order of renal function. If the total amount of water in the body is used as a basis of comparison in place of the surface area, then babies at birth still have a lower *GFR* than adults, but the *GFR* per unit of total body water reaches adult values in about two weeks. Thereafter, for the first year or so of life, infants have *higher* filtration rates than adults on this basis, which may be appropriate to the peculiarities of body composition and metabolism during infancy. Two reviews by McCance may be consulted for further information on the renal physiology of infants. [52, 53].

A filtration rate of over 100 ml. per minute seems large when compared with ordinary figures for the output of urine, but it does not seem so large in relation to the area of the filtering surface, which has been estimated to be from 1 to 2 square metres. Pappenheimer [64] has pointed out that filtration is much *more rapid through the glomerular* capillaries than through capillaries elsewhere in the body. The absolute rate of filtration per unit of hydrostatic driving force is also much larger than the

permeability to water of most cell membranes. This difference is in harmony with the current conception of filtration as a process which takes place through pores in the intercellular cement rather than through two layers of membrane and across the intervening cytoplasm of the endothelial cells.

VARIATIONS IN GLOMERULAR FILTRATION RATE

The following are some of the factors which have been found to influence the rate of glomerular filtration. (See a recent review by Brod [10] for more details of many of these.)

1) The rate of glomerular filtration may fall to very low values in shock or after severe haemorrhage, when the volume of circulating blood is grossly diminished.
2) Smaller reductions in glomerular filtration rate have been observed during the severe dehydration which follows depletion of the body's stores of extracellular sodium.
3) The glomerular filtration rate is reduced by muscular exercise, in rough proportion to its severity.
4) Hypophysectomy in experimental animals is followed by a diminution in glomerular filtration rate.
5) The glomerular filtration rate falls when the number of functioning glomeruli is reduced by progressive renal disease.
6) The glomerular filtration rate depends to some extent upon the basal metabolic rate It seems to be related particularly to the metabolism of protein, and has also been found to increase after taking food.
7) Diurnal variations have been described, the glomerular filtration rate tending to be somewhat lower by night than by day.
8) Alterations in blood pressure often produce smaller changes in filtration rate than might be expected Infusions of adrenaline may increase the arterial blood pressure and reduce the flow of blood through the kidney without altering the glomerular filtration rate. Since the volume of the kidney has been found to increase under these conditions,

adrenaline is believed to reduce the circulation through the kidney by a vasoconstrictor action primarily upon the efferent arterioles, which increases the filtration pressure at the same time.

9) There are indications that the rate of glomerular filtration follows alterations in the volume of circulating blood or of extracellular fluid within the physiological range.

10) It is generally accepted that the glomerular filtration rate is affected by the colloid osmotic pressure of the plasma proteins, although there seems to be hardly any experimental evidence on this point. Starling showed in 1899 that no urine was formed by the dog's kidney, presumably because glomerular filtration ceased, when the filtration pressure was reduced below the oncotic pressure of the plasma proteins by obstructing the ureter, and he suggested that the glomerular filtration rate should be proportional to the excess of hydrostatic pressure over oncotic pressure.[90] This reasonable hypothesis has never been proved experimentally. Indeed Homer Smith[85] has remarked "It seems to be generally true that changes in oncotic pressure, like changes in arterial pressure, are not directly reflected in changes in renal function because they are wholly offset by autonomous changes in the renal circulation, or by changes in the latter brought about by factors the nature of which remains unknown".

This stability of glomerular filtration has been put forward as one of its most characteristic features. The glomerular filtration rate seems to be a physiological constant, at least under basal conditions; but it must be remembered that the most reliable measurements require continuous infusions of inulin, and so cannot be made under conditions far removed from basal. The constancy of the glomerular filtration rate may have been overstressed formerly because it could only be measured satisfactorily when it was constant. A spectacular demonstration of the

variability of the human glomerular filtration rate was provided by a student whose inulin clearance increased to a peak value of 285 ml. per minute (more than twice its initial value) after the intravenous infusion of a 10% solution of sodium chloride. But all too little is known about variations in filtration rate over short periods of diverse physiological activity. Endogenous creatinine clearances do not seem able to yield much information on this point, for although they can be determined without restricting the activity of the subjects, they are most suitable for measuring average rates over long periods of time — if indeed they measure glomerular filtration rates at all. It may be concluded that extreme variations in glomerular filtration rate are not known to occur under physiological conditions. Glomerular intermittence of the kind familiar in the frog probably does not occur in mammals, with the possible exception of the rabbit, whose vasmotor activity and renal function are remarkably labile. Since mammals lack a renal portal circulation they cannot close down glomeruli without depriving tubules of blood. If resting glomeruli exist at all in mammalian kidneys, they are perhaps glomeruli whose efferent arterioles are dilated rather than glomeruli whose afferent vessels and capillary loops are constricted.

TUBULAR REABSORPTION

A glomerular filtration rate of 130 ml. per minute is equivalent to almost one half the volume of the kidneys per minute, and amounts to 187 litres in a day. This is more than a dozen times the total volume of the extracellular fluids, and it means that the amounts of a number of constituents of the plasma which leave the circulation every day in the glomerular filtrate are approximately as shown on the left in Table 1.

The quantities of the same substances which appear in the urine are shown on the right for comparison. It is clear that the tubules remove most of the glomerular filtrate formed, and

TABLE 1

A comparison of one day's glomerular filtrate and urine.

Substance	Amount in glomerular filtrate	Amount excreted in urine
Sodium	600 grams	6 grams
Potassium	35 grams	2 grams
Calcium	5 grams	200 mg.
Glucose	200 grams	Trace.
Urea	60 grams	35 grams
Water	180 litres	1.5 litres.

since it does not accumulate in the kidneys it must be reabsorbed and returned to the blood. In this way the tubules reabsorb 99% of the sodium and water, all the glucose, most of the potassium and calcium, and rather less than half of the urea of the glomerular filtrate. The main function of the tubules is therefore the *conservation* of the soluble constituents of the body. The first of the three principal functions listed on page 5 appears to be performed by the glomeruli, and the second by the tubules.

This sequence of glomerular filtration and tubular reabsorbtion may apply to a limited extent to the plasma proteins, for although it is often stated that the capsular fluid is a protein-free ultrafiltrate of the plasma, some of the specimens analysed have contained small amounts of protein, which might have been the result of trauma or might signify the normal presence of a quantity of protein near the limits of sensitivity of the methods employed to detect it If the capsular fluid contained as little as 20 mg of protein per 100 ml., which is less than the cerebrospinal fluid may contain, this would be small enough in comparison with the 6,000 to 7,000 mg. per 100 ml. in the plasma for the capsular fluid still to be regarded as an ultrafiltrate for most purposes, but it would have the important consequence that the daily excretion of over 30 grams of protein might be attributed to glomerular filtration alone It follows that even gross

proteinuria need imply no considerable alteration in the permeability of the glomerular membrane, and might be produced by the failure of a normal process of tubular reabsorption Dogs seem to have a renal threshold for plasma protein at about 10 g. per 100 ml., above which plasma protein appears in the urine. If mammalian glomerular fluids do contain small amounts of plasma proteins which are normally reabsorbed by the tubules, the tubular damage seen histologically in the kidneys of patients who have died with nephrosis might be the morphological counterpart of a disorder of function which had destroyed the normal capacity of the tubules to reabsorb protein, or it might be the result of reabsorbing unusually large amounts of protein to compensate for an increase in glomerular permeability But if proteinuria in nephrosis is a consequence of increased glomerular permeability, the wonder is that the amount of protein appearing in the urine is not much greater. And if mammalian capsular fluids do not *normally contain protein, the glomerular* capillaries which have in other respects a greater permeability than capillaries elsewhere must be the only capillaries in the body which are wholly impermeable to protein. This whole topic has recently been reviewed by Rather,[72] and Squire has discussed the variations in glomerular permeability which may be detected in nephrotic patients.[89]

Tubular reabsorption may be active or passive. Glucose is normally always present in lower concentration in the urine than in the blood, and it must be reabsorbed by an active process which can supply energy to transport it through the cells of the tubular epithelium. The reabsorption of urea, on the other hand, is regarded as a passive process in mammals, and to emphasise this it is usually spoken of as "back-diffusion". Unlike glucose, the reabsorption of which always takes place against a concentration gradient and is abolished by phlorizin, urea is always reabsorbed from a higher to a lower concentration. At high minute volumes the clearance of urea is independent of the minute

volume, and about two-thirds that of inulin; but when V is less than 2 ml. per minute, the urea clearance falls, and is approximately proportional to the square root of V. Hence most urea is reabsorbed when the urine is most concentrated and the flow along the tubules is slowest, — that is, when the concentration gradient from tubular lumen to blood is steepest and the time available for back-diffusion to occur is longest. Some experiments of Shannon showed that as the minute volume became larger and the concentration gradient diminished during extreme diuresis in dogs, the clearance of urea approached that of creatinine.[79] Dole has shown by calculations based upon a simple model system, that back-diffusion, determined by the concentration gradient and the time available for it, could account for all the observed relations between the minute volume and the clearance of urea.[18]

If the kidney is regarded as an organ with the primary function of excreting waste-products from the body, this combined mechanism of glomerular filtration and tubular reabsorption seems round-about and needlessly cumbersome. It may however be a legacy of the evolution of modern mammals from ancestors which dwelt in fresh water. Their problem must have been to get rid of the water which continually diffused into their more concentrated body fluids from diluter surroundings. Glomeruli provided an economical means of eliminating water at the expense of the mechanical energy of the heart. But though water might conveniently be eliminated in this way, it carried with it the essential solutes of the body as well as unwanted end-products, and there was need of a further mechanism to recapture those solutes which were required to sustain the life of the cells. Compared with animals which inhabit fresh water, the marine teleostean fishes are faced with the opposite problem of conserving water which their more concentrated habitat tends to abstract from them by osmosis; they have developed kidneys with relatively few glomeruli, and some have lost their glomer-

uli altogether. Similarly, the reptiles which colonized arid places and had need to conserve water, and the birds which have been developed from them, have kidneys with relatively few glomeruli. Their capacity to economize in water is assisted by the adoption of uric acid as an insoluble end-product of nitrogenous metabolism, which, unlike the extremely soluble urea, can be excreted without water, and whose insolubility has made possible the development of these species in water-tight (cleidoic) eggs. The modern mammal, which, *during its embryonic* development may be said, through the placenta, to have the use of its mother's kidneys, excretes its nitrogenous wastes as the highly soluble urea. It has therefore to lose water in order to excrete this in solution, and it still has a kidney with a large number of glomeruli. Unlike its early ancestors it has acquired the power to economize water by excreting a hypertonic urine. Water has been added to the list of substances which can be actively conserved by the tubules.

Four consequences of this round-about filtration-reabsorption mechanism of the mammalian kidney may be noted.

1) Since so large a proportion of the glomerular filtrate is reabsorbed, the reabsorbed fluid cannot differ greatly in average chemical composition from the filtrate, or from *the plasma except in protein content. It resembles the other* extracellular fluids of the body.
2) Relatively slight alterations in tubular activity may lead to large changes in the urine. If, for example, the tubules reabsorbed 1% less sodium than shown in Table 1, the amount in the urine would be increased by 100%.
3) Conversely, large alterations in the volume and composition of the urine may imply only small alterations in tubular activity. It follows that changes in the function of the tubules are relatively difficult to detect, and even more difficult to measure with any degree of precision. The limit of accuracy is determined by experimental errors involved

in measuring the glomerular filtration rate, about which there may be considerable uncertainty; and by the errors in *analysing the plasma, some constituents of which are present* only in small concentrations.

4) If "secretion" is taken to mean active transport through cells at the expense of metabolic energy made available within them, then most secretion in the kidney, unlike that in other glands, is directed "backwards", from duct to blood. The *urine is from this point of view a by-product, containing* chiefly substances left over from the real process of tubular secretion. This seems paradoxical if the kidney is regarded as an excretory organ, but it makes sense if the kidney is thought of as an organ whose main function is to safeguard the composition of the extracellular fluids by conserving the soluble constituents of the body. The tubular epithelium might even be described as the greatest of all the glands of internal secretion, for it secretes no less than 180 litres of extracellular fluid into the blood every day. The earlier statement that the reabsorbed fluid resembles the other extracellular fluids in composition hardly went far enough. The reabsorbed fluid is a most important source of all other extracellular fluids; and the renal tubules regulate the composition of the extracellular fluid by secreting it. To look upon the kidney in this way may seem topsy-turvy, but it is important for the avoidance of muddled thinking about renal function. One of its important consequences is that a diuretic which increases the output of urine without increasing the glomerular filtration rate is to be classed as an inhibitor, and not as a stimulant, of renal activity — at least of that kind of renal activity which depends upon work done by the secreting cells inside the kidney. The clinical observation that the failing kidney may first declare itself by producing too great a volume of urine is not paradoxical *when considered from this standpoint.*

III. TUBULAR SECRETION

The emphasis so far has been upon reabsorption as the major activity of the tubules — upon their conservative functions. The clearance of a substance which is reabsorbed by the tubules is less then the clearance of inulin determined simultaneously, or, as this is often expressed, the "Clearance Ratio" (Clearance of X/Clearance of Inulin determined at the same time) is less than unity. When such a substance is being excreted the urine contains less of it than the glomerular filtrate from which the urine is formed, so that the epithelial cells must remove the substance from the tubular fluid as it flows past them. Glucose, with a clearance ratio close to zero, sodium chloride, with a clearance ratio of about 0.01, often expressed as 1%; and urea with a clearance at ordinary minute volumes of about 60% of the filtration rate, are examples. There are other substances whose clearance ratios are *greater than unity. The urine contains more* of these substances than the glomerular filtrate from which it is formed, so that they must be transported from the plasma to the tubular fluid by the epithelial cells. *In so far as tubular excretion* of this kind takes place against concentration gradients, it is a process of active secretion, like the reabsorption of glucose, but directed outwards instead of inwards.

The entire urine of those animals whose kidneys possess no glomeruli must be excreted by the tubules, and aglomerular kidneys can excrete most of the familiar urinary constituents. Homer Smith[33] lists water, creatine, creatinine, urea, uric acid, magnesium, sulphate, potassium and chloride. Even the water appears to be excreted actively and not merely withdrawn passively from the cells by the osmotic activity of solutes secreted

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first, because urine may be secreted against a pressure several times greater than that of the blood perfusing the kidneys.

Tubular excretion of uric acid occurs in birds and reptiles, and tubular excretion of urea in frogs.

TUBULAR SECRETION IN MAMMALS

A *Physiological Substances*

Since the proximal convoluted tubules of mammalian kidneys have the same cytological structure as the tubules of aglomerular kidneys, and the proximal tubules are regarded as homologous in all vertebrates, the behaviour of the aglomerular kidney cannot but raise a suspicion that tubular excretion might occur in mammalian kidneys along with glomerular filtration and tubular reabsorption. Such excretion could never be detected with certainty unless it gave rise to a clearance greater than the glomerular filtration rate, otherwise the difference between the rate of filtration ($F . P_x$) and the rate of excretion ($U_x . V$) would merely under-estimate the rate of tubular reabsorption The possibility that most constituents of the urine are excreted by the proximal tubule as well as filtered through the glomerulus, and then largely reabsorbed from lower segments of the nephron, is therefore one which cannot readily be either proved or disproved. It may be necessary to postulate a "Three-component system" of this kind (filtration + tubular excretion + tubular reabsorption) to account for the excretion of potassium, which has a clearance in man sometimes greater, although usually less, than the clearance of inulin, but as applied to substances whose clearances are always less than the glomerular filtration rate, it is rather an unprofitable hypothesis so long as it leads to no unequivocal evidence for or against it.

The following are examples of physiological substances which have been found to be excreted by mammalian renal tubules.

1. N-methylnicotinamide. This is a metabolic derivative of nicotinic acid and of nicotinamide which is excreted by dogs

and may have a clearance up to three times that of creatinine.

2. Creatinine. Creatinine is derived from the creatine of the muscles, and may be actively excreted by the tubules of the human kidney, but perhaps only when its concentration in the plasma is artificially elevated. The discrepancy, apparent or real, between the handling of endogenous and exogenous creatinine by the kidneys has been discussed on page 19 in connection with the problem of finding substances whose clearances measure the glomerular filtration rate.
3. Potassium. This cation can sometimes be shown to be excreted by the tubules of men and dogs. Usually its clearance is only a fraction of that of creatinine or inulin, but clearance ratios greater than unity have been encountered with diseased kidneys, and with healthy kidneys under rather special experimental conditions, as for example
   i) after the administration of large amounts of the potassium salts of foreign anions.
   ii) during osmotic diuresis, and
   iii) when inhibitors of carbonic anhydrase have been administered during acidosis.
4. Ammonium. This ion occurs in the urine of many animals; no clearance can be calculated, because the ammonium in the urine is not derived from the blood, but from ammonia synthesized in the tubular epithelium. Most of the ammonia formed in the kidney is excreted in this way in the urine, but some enters the blood, and the venous blood from the kidneys contains more than the arterial blood, though both contain so little ammonia that sensitive methods of analysis are required to estimate it
5. Hydrogen ions. The mammalian renal tubule is able to excrete hydrogen ions as a means of acidifying the urine. Like the ammonium excreted by the kidneys, the hydrogen ions in the urine are not derived from the blood but are generated in the tubular epithelium. The excretion of acid,

of ammonium and of potassium will be discussed in more detail in a later chapter on the contribution of the kidneys to the maintenance of the acid-base balance of the organism.

It may be concluded that with the exception of hydrogen ions and ammonium, which are synthesized in the kidney, tubular excretion does not play an important part in the fabrication of normal mammalian urine. The excretory functions of the kidney are performed almost entirely by the glomeruli.

B. *Foreign Substances*

The kidney responds in a completely different manner to a number of foreign substances, which the tubules excrete with remarkable efficiency. This behaviour is the more striking because the kidney can hardly have encountered these substances during its long evolutionary history. Phenolsulphonephthalein (the indicator, phenol red, often abbreviated to *PSP* in clinical writings on the kidney) was among the first to be investigated It is a dye which is bound by plasma proteins, and is therefore only partially filtrable. About 20% of it is free when the plasma contains 1 mg. per 100 ml., yet when it is present at this low concentration its clearance in normal men is a little over three times that of inulin The urine therefore contains about 15 *times as much phenol red as the glomerular filtrate from which* it is formed, and the dye must be excreted by the tubules to a far greater extent than any normal organic constituent of the urine. When the concentration of phenol red in the plasma is raised, its clearance falls, a point which will be taken up later (p. 45).

There is a group of substances which are excreted even more completely than phenol red. Diodone, para-aminohippurate and penicillin have clearances in man which are between 600 and 700 ml. per minute, a little over 5 times the simultaneously determined clearance of inulin. As with phenol red these high rates of excretion are observed only when the concentration in the plasma is low. Diodone, (called diodrast in American publi-

cations) is 3,5-di-iodo-4-pyridone-N-acetic acid, and is used to allow the renal tract to be visualized or photographed radiologically. It is excreted in high concentration within a few minutes of its administration by intravenous injection, and the iodine which it contains makes it radio-opaque. It can also be used to measure the excretory capacity of the tubules, but for this purpose para-amino-hippurate, (PAH) which has the same clearance, is now preferred because it is less toxic, bound less by plasma proteins, and easier to estimate. The rapid excretion of penicillin by the kidneys serves no useful purpose except in the treatment of infections of the urinary tract. It is a source of inconvenience when systemic infections are being treated, because it is responsible for the need to administer large and frequent doses in order to maintain an adequate level of bacteriostatic activity in the blood.

There is a natural upper limit to the rate of excretion of any substance which is not synthesized within the kidney, it cannot exceed the rate at which the substance is brought to the kidneys by the arterial blood. Thus if the concentration in the renal arterial plasma is P, and the total rate of circulation of plasma through both kidneys is R, the rate of excretion UV cannot be greater than $R.P.$ If the whole of the substance reaching the kidneys in the arterial blood were transferred to the urine, we should have

$$UV = R.P. \qquad (3)$$

so that $$R = \frac{UV}{P} \qquad (4)$$

In words, the clearance of a substance which was completely removed from the plasma during one passage through the kidneys would be equal to the total rate of circulation of plasma through both kidneys. This is usually referred to as the "Renal plasma flow", or by the abbreviation "RPF". If U and P are in mg. per ml. and V is as usual in ml. per minute, R is also given

in ml. per minute. Since the blood is never in fact completely cleared by once circulating through the kidney, all actual clearances under-estimate the renal plasma flow.

Even if the cells lining the tubules were able to take all the diodone or PAH which reached them in the blood circulating through the intertubuar capillary plexus, and to transfer it to the urine, some of the test substances would remain in the blood emerging from the renal veins, because not all the blood which circulates through the kidneys is presented to the tubular epithelium; a portion of it perfuses non-secretory tissues within the kidney. The blood issuing from the renal veins can be sampled in animal experiments by direct puncture, and in man by means of a flexible catheter introduced into a vein in the arm, steered through the heart into the inferior vena cava, and finally directed into the renal vein. Analysis of blood obtained in this way while the the kidneys have been excreting diodone or p-aminohippurate has shown renal venous blood to contain only about one-tenth of the concentration present in the renal arterial blood, so that approximately 90% of the diodone or p-aminohippurate present in the arterial blood can be removed from it and transferred to the urine during one passage of the blood through the kidney. This result is generally expressed by saying that the "Extraction" is 90% complete, or that the "Extraction ratio" of the test substance is 90%. The remaining 10% which is not extracted must include both the substance left behind by incomplete clearance of blood presented to the tubular epithelium, and also that contained in the blood which perfuses non-secreting tissue. Since the sum of these is so little as 10% of the total, it follows that the tubular epithelium must remove these substances from the blood which perfuses it with remarkable efficiency.

There are two ways in which the rate of excretion of para-aminohippurate or diodone may be employed to determine the circulation rate of plasma through the kidneys.

1. It may be assumed that the tubular epithelium clears the test substance completely from all the blood which reaches it. This is tantamount to assuming that the extraction ratio for blood which perfuses secreting tissue is always 100%. The plasma clearance of p-aminohippurate or diodone is then determined, and equated with the "Effective renal plasma flow", which is, by definition, the rate of circulation of plasma through secretory tissue with its assumed extraction ratio of 100%. It is important to note that the effective renal plasma *flow determined in this way is not the same as* the total rate of flow of plasma through the kidneys, but it does represent the greater part of that total flow numerically, and the part which is physiologically most important This method does not require a determination of the extraction ratio, and since the extraction ratio can only be determined in man by submitting the patient or experimental subject to cardiac catheterization, the effective renal plasma flow is the one usually determined in practice.
2. The total rate of circulation of plasma through the kidneys may be calculated accurately and without any assumption about the completeness with which the tubular epithelium clears the blood which perfuses it if an extraction ratio is determined at the same time as the plasma clearance. For then

$$\text{True } RPF = \frac{\text{Clearance} \times 100}{\%\ \text{Extraction}} \quad \ldots\ldots\ldots (5)$$

Thus for example, if the extraction ratio is 88%, then the rate of excretion $U.V$ is 88% of the rate at which the test *substance arrives at the kidneys in the arterial plasma.* The plasma clearance is therefore 88% of the renal plasma flow, and $RPF =$ Clearance/0 88. It will be noticed that this procedure is equivalent to a straightforward application of *Fick's principle. It* does not depend upon any assumption about the completeness of clearance by the tubules, because

consumption, the arteriovenous difference in oxygen content of the blood flowing through the kidney is low, of the order of 1 ml. O_2 per 100 ml. of blood in man. The rapid renal circulation is the expression of services rendered to the blood by the kidneys, rather than of the reverse. From each 650 ml. of plasma which flows through them the kidneys form about 130 ml. of *glomerular filtrate. One-fifth of the plasma is therefore filtered* through the glomerular capillaries. Among other results of this reduction in the volume of the plasma without the loss of its proteins is an increase in colloid osmotic pressure to perhaps 40 mm. Hg., which may be a factor in bringing filtration to an end when 20% of the plasma has been converted into filtrate. It is unlikely to be the only factor, however, because even though the proportional increase in colloid osmotic pressure is greater than that in concentration, there should still be an ample head of filtration pressure; and sometimes a greater proportion of the plasma passing through the glomeruli is converted into glomerular filtrate. *The extent to which the plasma is concentrated* by glomerular filtration is thought to be determined largely by the relative vasomotor tone of the afferent and efferent arterioles. Hence the ratio of the inulin clearance to the clearance of diodone or of para-aminohippurate is frequently determined in studies of renal haemodynamics. This ratio of filtration rate to plasma flow is known as the "Filtration fraction", abbreviated to *FF*, and its normal value, as stated above is, about 0.2. Higher values are usually interpreted as indicating increased constrictor *tone of the glomerular efferent arterioles. The filtration fraction* is characteristically increased during infusions of adrenaline and decreased during hyperaemia of the kidneys. Reviews by Winton[108, 109] should be consulted for further information about the part played by physical factors in the working of the kidneys.

THE REGULATION OF THE RENAL CIRCULATION

The development of clearance methods for measuring the

allowance is made for the amount of test substance remaining in the renal venous blood. If, by way of illustration, one half of the tubule cells ceased to function, but there was no change in the amount of blood circulating through the kidneys each minute, the first method would show an effective renal plasma flow of half the normal value, which might be misinterpreted as a 50% reduction in the renal circulation rate. The second method would show that the extraction ratio had fallen, and would give the correct value for the renal circulation rate.

It should be pointed out that (5) is only an approximate formula because it neglects the fact that the excretion into the urine of some of the water which enters the kidneys in the arterial blood raises the concentrations of all solutes in the renal venous blood. This has the effect or reducing the apparent extraction ratio and makes the renal plasma flow calculated from it too large. When the extraction ratio is high, as it is with diodone or p-aminohippurate, the error from using equation (5) is small enough to be neglected A slightly more complicated formula developed by Wolf (Reference 112, p. 65) must however be employed to calculate the renal circulation rate from the excretion of substances like inulin or urea which have smaller extraction ratios.

THE CIRCULATION THROUGH THE KIDNEYS

The circulation rate determined in this way in man is found to average 650 ml of plasma per minute, which corresponds to a circulation rate through both kidneys of about 1,200 ml. of whole blood per minute. This rate is large, over two pints per minute; it is almost one quarter of the cardiac output at rest; about one quarter of the blood in the body passes through the kidneys each minute. So rapid a circulation is not required to supply the kidneys with oxygen Although the renal parenchyma is among those tissues which have high rates of oxygen

effect of foreign protein proved to be reproducible with other pyrogenic batches of inulin or with typhoid vaccine administered intravenously. The renal circulation rate was sometimes increased as much as 100%. Even if the chills, the headache and the rise of temperature associated with the reaction were prevented by the prior administration of amidopyrine, the renal circulation rate increased as usual The hyperaemia associated with reactions to pyrogens is not confined to the kidneys, for the cardiac output and the circulation through the liver also increase. A similar increase in renal circulation during pyrexia in dogs has been shown to be unaffected by denervation of the kidneys

A few drugs, such as derivatives of adenylic acid, and hydrazino-phthalazine hydrochloride (Ciba 5968, Apresoline) have been found to increase the circulation through the kidneys, but most reputed vasodilators do not Xanthine derivatives such as caffeine and theophylline seem only to produce a transient increase followed by a sustained decrease, and nitrites reduce the renal circulation rate

More factors are known which reduce the circulation rate through the kidneys than which increase it It is reduced during vigorous muscular exercise, during dehydration, when the volume of circulating blood is diminished by haemorrhage and shock, and also by pain and fright. Of pharmacological agents adrenalin reduces renal blood flow, but ephedrine does not. Posterior pituitary extracts, in physiological doses which produce maximal antidiuretic effects, do not reduce the renal circulation rate. Renin and angiotonin reduce the flow of blood through the kidney. The circumstances by which the renal circulation rate is reduced suggest that the kidneys provide a sort of circulatory depot from which the flow of blood may be diverted when other needs are temporarily more urgent than the production of urine. The actual volume of blood contained in their vessels is too small for the kidneys to function as a blood depot in the ordinary sense, but the closing down of their

circulation through the kidneys was a great advance. Formerly, because of the relative inaccessibility of the kidneys and their blood vessels, measurements could only be made upon animals after they had been submitted to considerable surgical trauma. A number of the earlier measurements were inevitably made upon animals suffering from shock, and the application of such methods to normal human subjects was unthinkable. Clearance methods enabled the circulation of blood through the kidneys to be studied in intact animals and in human patients and experimental subjects without the complicating effects of trauma or anaesthetics. Even with these methods however, emotional disturbances and effects of conditioned reflexes (to be discussed later, p. 37) cannot be avoided. Moreover by the very nature of the techniques, their application is limited to experiments upon relatively placid individuals under fairly quiet and non-strenuous conditions This perhaps partly explains why the renal circulation rate, like the glomerular filtration rate, has seemed to be rather stable.

The kidneys are richly supplied with sympathetic nerves, which are believed to be distributed mainly to the blood vessels, although there is some evidence that they also supply the tubular epithelial cells. There does not appear to be any normal vasoconstrictor tone, for the circulation through the kidneys does not increase when they are denervated. In fact few factors have so far been discovered which cause a large increase in the circulation of blood through the kidneys, and the first of these was discovered by accident. Although Homer Smith and his colleagues found that the circulation through the kidneys was not increased by some fashionable diuretics or by substances which were known to act as vasodilators in other situations, a batch of inulin used for the determination of glomerular filtration rates chanced to be contaminated with pyrogenic material, and during the febrile reaction which followed its administration the renal plasma flow was increased considerably for several hours. This

physiologists, and its *importance is at present hard to assess.* For fuller treatment of the problems which it raises the reader should read Trueta's book, Homer Smith's critical discussion[87, 88] and a recent article and a book by Sophian in defence of the shunt[87, 88].

One criticism of the thesis that the intra-renal diversion of blood described by the Oxford workers has an important part to play in mammalian renal physiology has been that drastic procedures, quite outside the physiological range, have been required to produce it, and that the effects have not always been reproducible even in rabbits. No compelling evidence seems to have been adduced that the blood can be diverted in a similar manner by physiological stimuli in any other mammal, with the possible exception of the rat. The rabbit, on which most of the work has been done, is notorious for the lability of its vasomotor and renal function. Moreover it is an animal associated by an unfortunate tradition with *conjurors*, and there is a suspicion abroad that it can be persuaded to yield any desired result by *choosing the right experimental conditions*.

Trueta and his colleagues regard the convoluted tubules of the juxtamedullary nephrons as embedded in and as sharing the same intertubular capillary plexus as those of the cortical nephrons, but they claim that the circulation through the juxtamedullary glomeruli and the vasa recta is a short path from renal artery to renal vein which by-passes the secretory tissue in the cortex. The operation of the shunt ought therefore to reduce the extraction ratio of diodone or of p-aminohippurate, for diversion of blood to the medulla should reduce the fraction of blood presented to the proximal tubular tissue which is believed to excrete these substances. The most convincing evidence of a short-circuiting of blood within the kidneys would, at first sight, be a reduction in the extraction ratio of say, p-aminohippurate, whilst at the same time the renal circulation rate was increased, or at least was not diminished, and the arterio-venous difference

circulation would allow a rapid circulation of blood to be maintained in other parts of the body with an appreciably smaller total cardiac output. The small reductions in renal circulation which occur under physiological conditions and after infusions of adrenalin or renin are achieved without reduction in glomerular filtration rate, and are therefore accompanied by an increase in filtration fraction. The manner in which these adjustments are brought about is not fully understood. They probably depend in part upon intrinsic mechanisms within the kidney, for they are not abolished wholly by denervation.

REDISTRIBUTION OF BLOOD WITHIN THE KIDNEYS

Apart altogether from variations in the overall rate of circulation, a redistribution of the flow of blood within the kidneys may occur. This phenomenon was described and illustrated in a book by Trueta and his colleagues [86] published in 1947. The fundamental observation was that certain drastic procedures such as strong electrical stimulation of the sciatic nerve, large doses of pitressin, or the application of tourniquets to the limbs, were followed in rabbits by a shunting of blood within the kidney, such that the outer two-thirds of the cortex became ischaemic, and the circulation was maintained through the juxtamedullary glomeruli. This diversion of blood from the cortex to the deeper parts of the kidney has come to be known popularly as the "Oxford shunt". The shunt is a short circuit because when it is in operation the time taken for blood to get through the kidney from the renal artery to the renal vein is reduced, as was demonstrated by radiographs taken in rapid succession after the introduction of radio-opaque material into the renal artery. The appearance of streaks of bright red arterial blood in the renal vein provided further evidence of the opening up of a short path through the kidney. The possibility that this mechanism plays an important part in regulating the function of the kidneys has not yet found favour with the majority of renal

will still have to be done to establish that the phenomenon which has been clearly described in rabbits occurs and is important in other mammals, including man.

THE CHARACTERISTICS OF TUBULAR SECRETION

It has already been stated that substances which are excreted so efficiently that they can be used to measure the renal circulation rate when their concentration in the plasma is low, are less completely cleared from the plasma when their concentration in it is higher. The influence of the concentration of a substance in the plasma upon its secretion by the tubules will now be considered in more detail as a preliminary to a discussion of the underlying mechanism of tubular secretion

If the rate of excretion *UV* of diodone or p-aminohippurate is plotted against the concentration of the substance in the arterial plasma, a curve is obtained of the general form illustrated in Figure 1. This curve, labelled *UV*, consists of three segments, of which the first and third are straight lines joined by a curve of transition (Segment 2) between the steeper slope of the first segment and the smaller slope of the third. The actual rate of excretion *UV* may be considered as made up of two fractions The smaller of these, at least at low plasma concentrations, is that due to glomerular filtration, which leads to excretion at a rate proportional to the concentration of the substance in the plasma, so long as the filtration rate remains constant. The straight line *F* in Figure 1 represents the fraction of the total excretion rate which is attributable to glomerular filtration. In actual experiments this line denoting the rate of excretion by the glomeruli is found to have the same slope as the third segment of the curve *UV* which shows the total rate of excretion by glomeruli and tubules combined. If for each value of the plasma concentration, *P*, the rate of excretion by the glomeruli (obtained from the straight line *F*) is subtracted from the total rate of excretion (curve *UV*) the dotted curve *T* is obtained. This curve shows

for oxygen was diminished. This combination of changes does not seem to have been demonstrated. On the contrary, Smith mentions observations made upon dogs during experimental shock, in which the renal circulation rate fell to as little as 3% of the control value, but in which the extraction ratio of p-aminohippurate was undiminished, and the kidneys appeared to be evenly perfused.

The negative evidence from clearances and extraction ratios may however be less conclusive than at first appears. A substantial fraction of the nephrons in human kidneys must possess juxtamedullary glomeruli. If the efferent blood from these juxtamedullary glomeruli by-passes the intertubular capillary plexus, so that the juxtamedullary nephrons are unable to excrete p-aminohippurate and diodone, how is the normal extraction ratio of 90% to be explained? Somehow or other the juxtamedullary nephrons must be able to participate in the tubular excretion of substances whose clearance measures renal plasma flow. It may be that the juxtamedullary nephrons secrete from the blood in the vasa recta to the fluid in the loop of Henle much as the cortical nephrons secrete from the blood in the peritubular capillaries into the lumen of the convoluted tubule. Another possibility is that solutes diffuse from the blood in the vasa recta and are carried to the epithelium of the convoluted tubules by ascending streams of interstitial fluid. Be this as it may, the important point is that if the tubules of juxtamedullary nephrons can excrete as well as those of cortical nephrons, no matter how they do it, shunting of blood within the kidney can not be disproved by the failure to demonstrate a depression of the extraction ratio of p-aminohippurate or diodone. But if the persistence of a high extraction ratio does not imply that the renal shunt is not in operation, it seems to imply instead that it is impossible to distinguish between the activity of juxtamedullary and cortical nephrons, and that it makes no difference whether the shunt is in operation or not. A great deal of work

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is raised, but more slowly. When still higher concentrations are reached (segment 3') the rate of excretion by the tubules becomes *constant, maximal, and independent of the plasma concentration.*

There is no difficulty in interpreting the straight segments of the curve of tubular excretion. In the range of rising plasma concentrations covered by the first segment, all the tubules must be excreting whatever is left behind in the plasma after the process of glomerular filtration In this range, all the tubules are capable of increasing their rate of transfer when the blood brings more of the test substance to them. In the range of concentrations covered by the third segment of the curve it is equally clear that none of the tubules can secrete any faster, however much more is offered to it by the plasma. The capacity of the transferring mechanism in all the tubules is now saturated. In the range covered by the transitional curve (segment 2') the plasma concentration is sufficient to saturate the transferring mechanism in some but not in all of the tubules. Those which are already saturated can secrete no faster in response to a further elevation of the plasma concentration, whereas the ones which are not yet saturated can do so. But as the plasma concentration increases, the proportion of tubules in which the secretory mechanism is saturated becomes greater, the number of tubules which can still increase their rate of transfer becomes less, and as it does so the curve flattens out to become horizontal when all are saturated The rate of total excretion (segment 3 of curve *UV*) continues to rise after the tubular secretion has attained its maximum rate because excretion by glomerular filtration can still be accelerated by a further elevation of plasma concentration. As the concentration in the plasma is raised, the clearance, which is *UV/P*, remains constant up to the point of inflection between the first and second segments of the curve of tubular excretion (Curve *C*) Thereafter it falls because the total rate of excretion no longer rises in proportion to the increasing plasma concentration. The clearance must continue to fall as the plasma

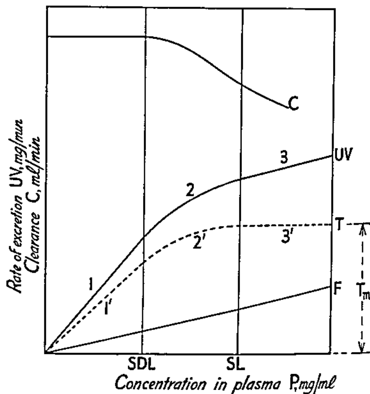


FIGURE 1. To illustrate influence of plasma concentration upon the excretion of substances with high extraction ratios

the rate of excretion by the tubules as a function of the concentration at which the substance is presented to them in the plasma. Like the curve of total excretion, the curve of excretion by the tubules has three segments. Along the first of these (1′), the rate of excretion is proportional to the concentration in the plasma. Over the transitional range indicated by segment 2′ the rate of excretion by the tubules still increases as the plasma concentration

is raised, but more slowly. When still higher concentrations are reached (segment 3′) the rate of excretion by the tubules becomes constant, maximal, and independent of the plasma concentration.

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level rises until, as glomerular filtration accounts for an ever increasing fraction of the total excretion, the clearance begins to approach the glomerular filtration rate as an asymptote. The clearance of a substance like phenol red which is bound to plasma proteins will of course fall below the glomerular filtration rate and will approach a fraction of the filtration rate which depends upon the proportion which is free in the plasma. Special names have been adopted for the plasma concentrations at which the two points of inflection occur. The concentration at which the first point of inflection is reached is known as the "self-depression limit" (*SDL*). This is the concentration at which the plasma clearance, hitherto constant, begins to be depressed by higher concentrations of the same substance, a phenomenon referred to as the "self-depression of the clearance". The concentration at which the second inflection occurs and the curve of excretion straightens out again, indicating that the transfer mechanism has been saturated in all tubules, is appropriately known as the "Saturation limit" (*SL*). When the plasma concentration is below the self-depression limit, the clearance is maximal, independent of plasma concentration, and measures renal plasma flow; the extraction ratio is also maximal. But extraction becomes less complete at concentrations above the self-depression limit, so that measurements of effective renal plasma flow, which do not require separate determinations of the extraction rate, can only be made by working at plasma levels below the self-depression limit. At higher levels the true renal plasma flow can still be estimated if the extraction ratio is determined in addition to the clearance. At plasma concentrations above the saturation limit, the rate of tubular excretion ($UV - FP$) becomes constant, maximal, and independent of the plasma concentration The value of ($UV - FP$) determined under these conditions is a measure of the total secretory capacity of the entire mass of tubular tissue in the two kidneys. It is denoted by T_m, with a further subscript to indicate the substance

to which it refers, e.g. T_{mD} for diodone or T_{mPAH} for para-aminohippurate. These are commonly spoken of as the "diodone T_m" or "PAH T_m". Sometimes this quantity is referred to as the "Tubular excretory mass", which might appear to suggest that all tubular tissue is equally efficient in the process of excretion, and although this may be true in health, it ought not to be assumed when the kidneys are diseased. It is safer to speak of "maximal tubular excretory capacity".

Thus a determination of the rate of excretion of a substance like diodone or p-aminohippurate may be used to give two quite distinct kinds of information about the kidney. By working with small doses and keeping the concentration in the plasma below the self-depression limit, a clearance may be determined which is a measure of the renal circulation rate. By using large enough doses to give plasma concentrations above the saturation limit, and by determining the glomerular filtration rate at the same time as the clearance of diodone or p-aminohippurate, the maximal tubular excretory capacity can be determined with the same test substance. A few actual figures may add reality to this rather abstract exposition. Diodone in man reaches its self-depression limit at a concentration of 5 mg. of iodine per 100 ml. of plasma, and its saturation limit at 40-50 mg. iodine per 100 ml. plasma. The diodone T_m is approximately 50 mg. iodine per minute, equivalent to approximately 0.2 mM diodone /min. Other excretory T_m values are 70-80 mg. (0.4 mM) per minute for p-aminohippurate, 16 mg. (0.14 mM) per minute for creatinine and 36 mg. per minute for phenol red.

The difference in plasma concentration between the self-depression limit and the saturation limit depends upon the dispersion of excretory capacity between the different tubules — it is a measure of the inequality of the members of a population of more or less similar units. One might venture to speculate that the first tubules to be saturated would be those of juxta-medullary nephrons. With their shorter length of secreting

epithelium, and possibly the less effective perfusion of their secreting tissue by blood, these should only equal the cortical nephrons in secretory activity at plasma concentrations below the self-depression limit, and should soon cease to respond to higher concentrations with a greater output. The operation of the renal shunt should not affect the extraction ratio at low plasma concentrations, but it might do so at concentrations above the self-depression limit.

Tubular excretion has been treated at so great a length not because of its importance in the daily work of the kidney, which appears to be trivial, but because by exploiting the excretion of foreign substances, physiologists have gained so much information about what goes on beneath the capsule. A little must be added about the influence of the concentration of glucose in the plasma upon its reabsorption by the tubules, for this is an everyday example of tubular secretion, although in the opposite direction Glomeruli and tubules co-operate in the excretion of p-aminohippurate and diodone, which are excreted partly by the tubules and partly by glomerular filtration. In their handling of glucose the glomeruli and tubules work in opposition, for at normal blood sugar levels, the glucose which enters the nephrons in the glomerular filtrate is removed by the tubular epithelium and restored to the blood.

The rate at which glomerular filtration delivers glucose to the tubules is $F \, . \, P_g$, where F is the glomerular filtration rate and P_g the concentration of glucose in the plasma; it is therefore proportional to the plasma concentration if the glomerular filtration rate remains constant. If the concentration in the plasma is gradually raised by infusing glucose into a vein, at what corresponds to the self-depression limit of diodone or p-aminohippurate some tubules begin to receive glucose faster than their epithelium can reabsorb it, and glucose begins to appear in the urine. The critical plasma concentration at which this occurs is known in this case as a "Threshold of appearance" or simply as

a "Threshold". It is important to note that the threshold does not necessarily indicate a critical concentration, but a critical *rate of delivery of glucose to the tubules, and that this depends* upon the filtration rate F as well as upon P_g. Even if the maximal reabsorptive capacity of the tubules is constant, the threshold plasma concentration must vary inversely with the glomerular *filtration rate, and will not be constant unless this is also fixed.* Patients in diabetic coma with blood glucose concentrations of several hundreds of mg per 100 ml sometimes excrete no glucose in their urine because severe dehydration has so far reduced *the glomerular filtration rate that the tubules can still reabsorb* all the glucose which reaches them Glycosuria may occur without hyperglycaemia as well as hyperglycaemia without glycosuria When the tubular epithelium is poisoned with phlorizin, its capacity to reabsorb glucose is impaired, and glucose appears in the urine even though the blood level and the glomerular filtration rate may be normal. The reabsorptive capacity of the tubules is subnormal in some otherwise healthy individuals, who excrete glucose in their urine although their blood sugar and glomerular filtration rate are both normal. The only importance of the condition, which may be familial or may occur as an incident during pregnancy, and is known as renal glycosuria, is that it may be confused with the much more serious diabetes mellitus unless the blood is examined as well as the urine. Tubules normally begin to be saturated in man when the *concentration of glucose in the plasma is elevated to about* 180 mg. per 100 ml. At a normal rate of glomerular filtration the tubules as a whole are then reabsorbing 1 8 x 130, or about 230 mg. of glucose per minute. As the concentration of glucose in the plasma is raised above the threshold, more and more tubules become saturated, until a saturation limit is reached above which the rate of reabsorption ($F P - U V$) becomes *constant and maximal.* Dogs, like men, show a saturation limit above which the rate of reabsorption of glucose by the tubules

ceases to increase as the concentration in the plasma is elevated still further, but cats do not.[22] In man and the dog, therefore, but not in the cat, a reabsorptive T_m, or glucose T_m, T_{mG}, can be defined, which measures the maximal reabsorptive capacity of the aggregate of tubular tissue. The value of the glucose T_m in man is about 350 mg. per minute, or almost 2 mM. per minute.

The maximal tubular secretory capacities measured by the excretion of p-aminohippurate or by the reabsorption of glucose are relatively constant and reproducible in repeated determinations in the same healthy individual. There is a somewhat greater variability between different individuals within a species, and there are naturally much larger differences between members of different species. A large amount of comparative data has been assembled in Chapter XVII of Homer Smith's book.[85]

There is an important difference between the maximal tubular secretory capacity measured with diodone and that measured with glucose. Diodone can be excreted by all tubules which are perfused with blood, whereas glucose cannot be reabsorbed unless it has previously gained entry to the nephrons by glomerular filtration Hence the diodone T_m is a measure of all the tubular tissue in the kidneys which is perfused *with blood*, whereas the glucose T_m takes account only of tubules attached to normally functioning glomeruli. If some disease process destroys glomeruli but spares the tubules to which they were attached, converting them into aglomerular tubules, the glucose T_m will be depressed but the diodone T_m may still be normal. On the basis of comparisons such as this Homer Smith has developed "saturation methods" whereby it is possible to perform a sort of physiological dissection of the kidneys of a human patient without seeing or touching them.[84]

THE MECHANISM OF TUBULAR TRANSFERRING PROCESSES

There is little that can be said with any confidence about the underlying mechanism of tubular secretion, for hardly anything

is known about it. The secretion of inorganic ions will be discussed in connection with the transport of water and the regulation of acid-base balance. The following is a brief account of the secretion of organic compounds which may be supplemented by reference to reviews by Beyer [5] and Taggart. [91, 95]

The secretion of organic compounds by the tubular epithelium possesses the following four characteristics:—

1. It has a limited capacity
2. It is suppressed by poisons.
3. It requires a source of energy, for many of the substances which are secreted are transported against concentration gradients.
4. It exhibits competitive inhibition in the sense that mutual interference can be demonstrated between the transport of different substances simultaneously. For example the four substances, penicillin, phenol red, diodone and p-aminohippurate interfere with each other's excretion. If the conditions are established for measuring the excretory T_m of any one of these substances, and another is then added to the blood, the tubules commence to excrete the second substance, and the rate of excretion of the first falls below its T_m Similar competition has been described for reabsorption, between glucose and other sugars, between leucine and isoleucine, and between the basic amino-acids arginine, histidine and lysine Neither leucine nor isoleucine competes with any of the basic amino-acids, and glycine does not compete with any of these five amino-acids, but competes with creatine. Competition has even been observed between the excretion of some substances and the reabsorption of others; for example, the reabsorption of glucose and of ascorbic acid may be depressed when large amounts of p-aminohippurate are being excreted.

These examples of competitive inhibition form too heterogeneous a collection to be covered by a single explanation. They

might arise from competition for a common chemical mechanism or pathway of excretion — an enzyme or a carrier substance common to several processes; or from competition for available energy. Competition for excretion between phenol red, diodone, p-aminohippurate and penicillin is probably competition for a common mechanism. These substances may all be excreted by the epithelium of the proximal convoluted tubule, because only the proximal tubules secrete phenol red in tissue culture; and the competition which can be demonstrated between phenol red on the one hand, and p-aminohippurate and diodone on the other, suggests that they are all excreted by the same cells. Competition within this group is, as Beyer pointed out, analogous to the competition of two substrates for oxidation by an enzyme; the maximum rate of oxidation of either when it is sharing the enzyme with the other is less than its maximal rate when present alone. When two members of this group compete for maximal tubular excretion, it may be presumed that the transport system remains saturated, but that its capacity has to be shared by the competing substances. Competition for reabsorption between sugars or between amino-acids is probably of the same nature. It is interesting to note in this connection that the T_m values of penicillin, diodone and p-aminohippurate are of the same order of magnitude when expressed in mM. per minute. I am indebted to Prof G. M. Bull for drawing my attention to this piece of additional evidence suggesting that the three substances are excreted by a common mechanism.

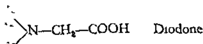
Enzymes can also be inhibited competitively by substances which resemble their natural substrates sufficiently to combine with them, but not sufficiently to undergo the reactions catalysed. These substances compete with the normal substrate for possession of the active groups of the enzyme molecules, and since they are not removed by reacting, they are more powerful inhibitors than competing substrates which merely divert some of the catalytic activity of the enzyme from other substrates

without reducing its total capacity. Beyer suggested that this type of inhibition had its counterpart in the action of carinamide on renal tubular secretion. Carinamide is a drug which was developed to reduce the excretion of penicillin by the kidneys so that adequate therapeutic levels could be maintained in the blood with smaller doses. Carinamide depresses, and in adequate doses may completely suppress, the tubular excretion of diodone, p-aminohippurate and phenol red as well as of penicillin, yet it did not at first appear to compete with them for excretion in the same way as they compete with each other, because its clearance suggested that it was excreted by glomerular filtration only. Although therefore carinamide seemed to provide an example of competitive inhibition by a substance which was not itself transported, it is not immediately obvious how a substance which does not enter the tubule cells can interfere with reactions which take place inside them, and subsequent work has disclosed a considerable binding of carinamide by plasma proteins, so that its clearance, which is of the same order as that of creatinine in the dog, indicates that it is excreted by the tubules.

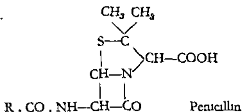
Mutual interference between the excretion of one substance and the reabsorption of others is perhaps most likely to be an example of competition for available energy. But it is difficult to say anything definite about this type of interference, for glucose and p-aminohippurate have been found to react with each other and form a readily hydrolysable complex, and this fact complicates the interpretation of experiments which appear to demonstrate that the excretion of p-aminohippurate interferes with the reabsorption of glucose. Ascorbic acid is ordinarily handled by the kidneys in such small amounts that its transport can hardly make a major claim upon supplies of energy.

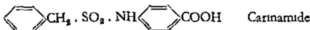
The failure of tubular function which follows poisoning with cyanide is presumably a result of cutting off supplies of the energy required by transporting mechanisms. Phlorizin, which suppresses both the reabsorption of glucose, and the excretion

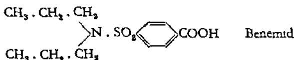
essential phosphorylating reactions. The energy which is released from substrates by oxidation is not in general used directly in biological systems, but is believed to be utilized primarily for the synthesis of labile phosphoric esters which contain what have come to be known as "high-energy" phosphate bonds. These are not extremely stable bonds as the name suggests; in fact they are extremely easily broken. They have two peculiarities, that when they are broken by hydrolysis the amount of free energy degraded and released as heat is unusually large, of the order of 12,000 cal./Mol, compared with 3,000 cal. for hydrolysis of ordinary phosphoric ester links; and that when they participate in chemical reactions a considerable part of this energy need not be degraded, but can be employed with the catalytic assistance of suitable enzymes to perform synthetic operations for which a supply of energy is required Compounds like adenosine-triphosphate and creatine-phosphate, which contain "high-energy" bonds of this kind, appear to serve as intracellular stores of available energy which may be utilized for most of the energy-requiring purposes of the cells Cellular activities which depend upon a source of energy may therefore be suppressed by inhibiting the respiration upon which the supply of energy ultimately depends, by preventing the energy released in respiration from being used to synthesize compounds containing "high-energy phosphate bonds", or by inhibiting the reactions whereby the energy stored in these compounds is released and applied Beyer pointed out that carinamide, and also the related compound benemid, can depress the tubular secretion of phenol red or of p-aminohippurate in concentrations which are too small either to inhibit the uptake of oxygen, or to uncouple phosphorylation from oxidation, so that these inhibitors might act by interfering with the utilization of energy in the cell. They have since turned


N—CH₂—COOH
Diodone


CO—NH—CH₂—COOH
p-aminohippuric acid

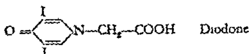

CH₃ CH₃
S—C
CH—COOH
CH—N
R . CO . NH—CH—CO
Penicillin

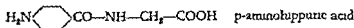

CH₂ . SO₂ . NH
COOH
Carinamide

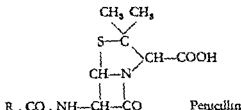

CH₃ . CH₂ . CH₂
N . SO₂
COOH
CH₃ . CH₂ . CH₂
Benemid

nism could account for the dependence of tubular transport upon energy stored in phosphate compounds like adenosine triphosphate, as well as for acetate as a limiting factor in the rate of transport, because adenosine triphosphate is required for the synthesis of acetyl-coenzyme A in the cells. It might also account for the fact that these foreign substances are excreted at all, for metabolites with carboxyl groups presumably participate in the normal metabolism of the renal cells, and the foreign substances may, so to speak, get caught up by their carboxyl groups in chemical machinery which is always running for some quite other purpose But although a carboxyl group may be necessary to gain entry into the metabolic conveyor which leads to excretion, it cannot be sufficient, for many more substances possess carboxyl groups than are secreted by the tubular epithelium. The carboxyl group may provide a point of attachment, but other structural features of the molecule must also fit into the metabolic machinery in a still unknown manner. No simple formula can adequately represent the structure of phenol red, but it is of interest to compare the structures of diodone, p-aminohippuric acid and penicillin with those of the two inhibitors, carinamide and benemid.

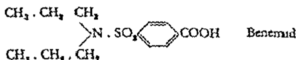
The problem remains how far it is justifiable to argue from accumulation by slices *in vitro* to tubular excretion *in vivo*. These processes appear to have something in common, for not only does the addition of acetate to the medium stimulate the accumulation of p-aminohippurate by kidney slices, but the infusion of acetate after conditions have been established for the determination of $T_{m_{PAH}}$ in the dog and also in man has been shown to increase the maximal tubular excretory capacity by as much as 100%. Moreover 2,4-dinitrophenol which inhibits accumulation by tissue slices *in vitro* also inhibits the tubular excretion of p-aminohippurate, diodone and phenol red in the dog; although curiously it does not depress the reabsorptive T_m of glucose. It cannot be supposed however that accumulation is a necessary











part of any possible process of tubular secretion. There seem to be three major possibilities: i) The substance being excreted is actively transported into the epithelial cells through the basal poles, thus building up a high concentration inside the cells, from which it can diffuse passively across the cell membrane at the opposite pole into the lumen of the tubule. Accumulation is an essential part of this mechanism ii) The substance is actively transported outwards across the membrane at the luminal pole of the epithelial cells, and diffuses into the cells passively

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fluid by reabsorption of water occurs after the substance has been added to it. Just what concentration would have to be built up in the cells is hardly calculable, but a mechanism which requires that substances being transported in both directions through the epithelial cells must be held in high concentrations inside them seems to leave too little room for the normal constituents of the cytoplasm, and to carry too great a risk of interference with essential metabolic processes within the cells by high concentrations of substances in transit. These difficulties might become less serious if future work confirmed a suggestion that the osmotic pressure of the intracellular fluids of the kidney is substantially greater than that of the plasma, but this question, which will be referred to again, is still unsettled. Such evidence as there is rather favours the second mechanism, and a mode of secretion which ensures that substances in transit are present in low concentrations in the cytoplasm seems to have obvious advantages. Fragments of mesonephric tubules of the chick form cysts in tissue culture; if these are supplied with oxygen and phenol red is added to the medium, the dye is secreted into the fluid inside the cysts. It is not however accumulated within the cells, which remain uncoloured; and dye introduced into the lumen of the cysts by micro-injection also fails to enter the cells, which argues against free diffusion across their luminal poles. Moreover in two recent studies by chemical and micro-radiographic methods the accumulation of diodone within the tubule cells during its excretion by the rabbit was demonstrated,[25] but only when large doses were employed and the transport system was saturated.[42] Here again it appears that accumulation within the tubule cells did not play an essential part in the mechanism of secretion. A number of observations have been made of the rate of excretion of substances like phenol red and p-aminohippurate while their concentration in the plasma was rapidly changing. The time relations between changes in excretion and changes in plasma concentration make it improbable that sub-

from the blood in the intertubular capillaries. Accumulation plays no part in this process, indeed the concentration of the *substance being excreted must be kept below that in the plasma* These mechanisms are not mutually exclusive for they might exist in combination, with active transport taking place across the membrane at both ends of the cells; some substances might be handled by one, and some by the other mechanism iii) The substance is converted by an enzyme system located at the basal pole of the cell into another form, for example a phosphoric ester, so that the concentration of the free substance is kept low just within the cell membrane, thus maintaining a steep concentration gradient across the membrane, down which the free substance can diffuse passively into the cell. Another enzyme system is required at the luminal pole of the cell to release the original substance in high concentration and allow it to diffuse into the lumen of the tubule. Transport of the phosphorylated complex across the cell might occur by diffusion from a high concentration in the region of its formation to the lower concentration produced by its decomposition at the opposite pole. It has been supposed that glucose is transported in this way across the intestinal mucosa and the renal tubular epithelium, but a recent study with isotopic tracers revealed that the phosphate radicals of phosphoric esters of glucose in the cat's kidney exchanged with *extracellular inorganic phosphate so slowly* as to suggest that phosphorylation can play no part in the reabsorption of glucose. [19]

Studies of the accumulation of metabolites by slices *in vitro* may only serve as valid models of the process of tubular secretion if this is carried out by the first of the three hypothetical mechanisms outlined above. This mechanism requires a high concentration of each substance which is being secreted (in either direction) to be maintained inside the tubular cells, although this concentration need not be as great as the concentration finally reached in the urine, because further concentration of the tubular

fold by the reabsorption of two-thirds of the water.[102] The concentration of chloride at the end of the proximal tubule was about 40% higher than in the plasma This was attributed partly to a Donnan distribution of ions and partly to replacement by chloride of bicarbonate which had been reabsorbed. As in the frog, there was no change of pH and no change in osmotic pressure in the proximal tubule, so that water and solutes must have been reabsorbed together in proportions to make a solution isotonic with the plasma; and since sodium salts are responsible for the greater part of the osmotic pressure of the plasma and of the glomerular filtrate, it follows that water and sodium salts must have been reabsorbed together in approximately their proportions in the extracellular fluids It has already been pointed out on other grounds (p 26) that the composition of the reabsorbed fluid as a whole cannot be very different from that of protein-free plasma The equality of osmotic pressure between the plasma and the tubular fluid, and therefore also between these two and the reabsorbed fluid, was confined to the proximal parts of the nephron Hypotonic fluid was found in the distal tubule of the rat, so that the fluid reabsorbed by the distal tubular epithelium must have been more concentrated than the plasma, and the epithelium of this part of the nephron must be able to reabsorb water and solutes independently. These experiments also showed that hydrostatic pressure was high in the proximal part of the nephron, and low in the distal tubule

A division of labour between different segments of the nephron is also indicated by work of a completely different kind In ordinary histological sections the tubules are most often seen cut across in transverse section; it is not certain to which part of the nephron any given cross-section belongs, and the tubules of one nephron cannot be distinguished from those of others. Jean Oliver has perfected a technique whereby whole nephrons can be isolated, stained in the usual manner, or treated by more subtle histochemical methods, and mounted on slides so that

stances undergoing tubular secretion are stored in the cells to an appreciable extent.

It must be concluded that it is not yet possible to give a detailed account of the metabolic processes employed by the epithelial cells to transport organic compounds between the blood and the urine.

LOCALIZATION OF FUNCTION IN THE TUBULE

Proximal tubule

Hitherto the renal tubules have been considered as a whole and no attempt has been made to assign particular sites to a variety of tubular functions. There is however evidence of a division of labour between different parts of any one tubule. Besides establishing the composition of the glomerular filtrate, the analysis of small samples obtained by puncturing the nephrons with quartz micropipettes yielded information about the composition of the tubular fluid at various stages on its journey towards the collecting ducts. By withdrawing fluid from different points along the tubules of the frog and Necturus, Richards and his colleagues found that glucose was reabsorbed in the proximal tubule; there was normally none left in fluid which had travelled half-way along this segment. [75] If its reabsorption was suppressed by phlorizin, glucose became more concentrated instead of disappearing from the tubule, thus providing an indication that water was reabsorbed. No change in osmotic pressure occurred throughout the length of the proximal tubule, so that the reabsorption of water must have been accompanied by a corresponding reabsorption of salts or other solutes. Osmotic pressure and pH remained constant until the distal tubule was reached. Here the fluid became acid, and it also became hypotonic, indicating a disproportion between the reabsorption of solutes and of water.

Walker and co-workers found similarly in the rat that all the glucose of the glomerular filtrate was reabsorbed from the proximal tubule, and that creatinine was concentrated three-

It may be concluded that, apart from its more spectacular occasional function of excreting foreign substances like diodone, the everyday activity of the proximal tubule is the reabsorption of the glucose and most of the water, salts, and amino-acids which were contained in the glomerular filtrate, whilst the substances to be excreted are retained within its lumen. The proximal tubules reduce the bulk of fluid in the nephron by a process which might be described as "Routine conservation".

Loop of Henle

After the proximal convoluted tubule comes the loop of Henle, the function of which is one of the mysteries of renal physiology. It was once suggested that the loop is the site of the concentrating process which enables mammals to conserve water by excreting a urine hypertonic to their plasma This suggestion was based upon studies of the comparative morphology of the kidney, which revealed a striking correlation between the presence of the thin limb, which is the characteristic part of the loop of Henle, and the ability of the kidney to elaborate hypertonic urine under the influence of an antidiuretic hormone secreted [illegible]

concentration is carried out there The loop of Henle might perform some preparatory function which is necessary to enable a more distal segment to concentrate the urine. The thin limb does not present the appearance of a secreting epithelium and doubts have been expressed whether it could perform the osmotic work required to abstract water from the urine and make it concentrated. This argument has little weight in itself, for the chloride-secreting epithelium in the gills of certain fishes is also thin, but there is no doubt that it performs a great deal of secretory work. The proposal that the urine is concentrated by a process of ultrafiltration through the epithelium of the thin

they can be examined under the microscope from glomerulus to collecting duct in continuity. This technique has disclosed that the division of labour between different parts of the same tubule may be so sharply demarcated that even adjacent cells often seem to be transporting quite different substances. Two interesting results of these investigations may be mentioned as examples They have shown by the appearance of droplets in the cells that the middle third of the proximal tubule in rats can reabsorb proteins such as haemoglobin, egg albumin, and proteins which have been coloured with dyes. They have also shed light on the mechanism of tubular saturation in the reabsorption of glucose, for when the amount of glucose entering the tubule is increased, the point at which reabsorption is completed shifts down the tubule. By sufficient loading with glucose it may be shifted into the distal half of the proximal tubule, the cells of which take up the task of reabsorption, and become loaded with glycogen. These cells seem able to take up glucose from the lumen when it reaches their level of the nephron, but unlike the cells of the proximal tubule whose accustomed task it is to reabsorb glucose, they do not appear to get rid of it so readily into the blood. Deposits of glycogen are also found in the distal part of the proximal tubule in clinical and in experimental diabetes mellitus. If the length of proximal tubule required for complete reabsorption of the glucose of the glomerular filtrate is proportional to the amount delivered per unit time, and so to the concentration of glucose in the plasma, nephrons will become saturated at blood sugar levels which are inversely proportional to the lengths of their proximal segments. The interval of plasma concentration which separates the threshold of appearance from the saturation limit might therefore reflect the variability in length of the proximal segments of different nephrons. Further details of this fascinating technique for the investigation of the cytology of the kidney, and of the results which have been obtained with it may be found in articles by Oliver.[61, 62]

assist the bulk-reducing function of the proximal tubules by a further, entirely passive stage of reabsorption of water and salts, which would require no secretory work from the epithelium, and would be an economical method of returning a fraction of the glomerular filtrate to the blood. The attainment of osmotic equilibrium between the fluid passing through the thin limb and the blood in the vasa recta would moreover ensure that a fluid of uniform osmotic pressure was delivered into the distal tubule.

If the loops of Henle do function in this way, there must be one very important difference between their epithelium and the glomerular filtering membrane. If these two membranes had the same permeability there is no reason why the whole of the glomerular filtrate should not return from the loops of Henle to the blood. The epithelium lining the thin limb must display a selective impermeability for substances of low molecular weight which is quite absent from the glomerular capillaries, it must indeed be a most peculiar membrane, for it must be almost "urea-proof". Back-diffusion of urea does occur, as has already been mentioned, but urea never returns to the blood to the same extent as water and sodium salts, and other organic solutes do not normally seem to return to the blood by back-diffusion to any appreciable extent. Urea is the most freely diffusible of all the solutes of the body; it is not supposed to be excluded from any territory, and its volume of distribution can be used to determine the total amount of water in the body. Free diffusibility through cell membranes everywhere inside the body is a valuable property of a substance which serves as an end-product of protein catabolism, for it minimizes osmotic disturbances of the water balance of the cells But the end-product has to be excreted, and the renal tubules must either excrete it actively or be able to restrict its free diffusion. Of all animals examined only the frog shows evidence of tubular excretion of urea, though the marine elasmobranch fishes, which employ urea to increase the osmotic pressure of their body fluds to balance the

limb by hydrostatic pressure has sometimes been made[95] but is ruled out by the consideration that to produce urine of a total concentration of 1.3 osmols per litre when the concentration of the plasma is 0.30 osmols per litre would require a pressure difference of 22.4 Atmospheres, or about 17,000 mm. of mercury. There is moreover an obvious disadvantage in concentrating the urine so far from the collecting ducts. The further upstream that the urine is made concentrated, with a proportionate reduction in the volume of fluid in the lumen of the tubule, the slower is the flow beyond the site of concentration, and the longer the time spent by concentrated fluid in contact with the epithelium Concentration in the loop of Henle would leave the constituents of the concentrated urine a large area of epithelium through which to diffuse back passively into the blood, and would allow a long time for them to do so. It is a fundamental property of the kidney that the greatest concentration of the urine can only be attained when the flow is slowest, which suggests that back-diffusion does not seriously interfere with the concentrating process, and that this is a late, if not the last operation performed before the tubular fluid enters the bladder as urine. The finding of hypotonic fluid in the distal tubule when the bladder urine was hypertonic also suggests that concentration was carried out far downstream in the nephron.[102] The anatomical relations of the thin limbs appear to allow for a free exchange of diffusible substances between their contents and the blood in the vasa recta. The arrangement is in some ways analogous to that in the glomeruli, but the physical forces are orientated to move filtrable substances in the opposite direction, for in the glomeruli the excess of hydrostatic pressure is on the same side of the membrane as the blood. Hydrostatic pressure is uncertain in the vasa recta, but is known to be high in the proximal convoluted tubule, and the colloid osmotic pressure of the blood in the vasa recta has been raised by the loss of an almost protein-free glomerular filtrate. The loops of Henle might therefore

assist the bulk-reducing function of the proximal tubules by a further, entirely passive stage of reabsorption of water and salts, which would require no secretory work from the epithelium, and would be an economical method of returning a fraction of the glomerular filtrate to the blood. The attainment of osmotic

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total concentration of the sea-water in which they live, have a special segment devoted to its reabsorption. The mammalian kidney, with its filtration-reabsorption mechanism, must restrict the diffusion of urea through all parts of the nephron where fluid is reabsorbed. It may not be an unreasonable guess that the loop of Henle serves to reduce further the bulk of fluid issuing from the proximal tubule; to allow the water and salts in the fluid which remains to come into equilibrium by diffusion with the blood in the vasa recta; but at the same time to retain the organic constituents of the urine within its lumen, for unless these were left behind the kidneys must fail in their excretory function.

Distal tubule

The division of labour proposed so far leaves to the distal tubule the intricate task of adjusting the final composition and concentration of the urine. Excretion by glomerular filtration and reabsorption by the proximal convoluted tubule and the loop of Henle seem to be relatively invariable. The distal tubules must employ selective processes, capable of fine adjustment, and which it would be uneconomical to apply to the whole bulk of the glomerular filtrate. It is difficult to conceive how these selective processes could be regulated if the fluid upon which the distal tubules had to operate varied in total concentration or in composition. This may be a further reason for preferring the view that the loops of Henle serve to ensure that the fluid entering the distal tubules is always isotonic with the plasma, to the view that they concentrate this fluid on some occasions under the influence of the antidiuretic hormone. A considerable part of the remaining sections of this book will be devoted to regulatory functions which are customarily attributed to the distal tubules

IV. THE EXCRETION OF WATER AND SODIUM

The composition and volume of the fluid entering the distal tubules of the human kidney cannot be known with certainty, but this fluid is likely to have the same osmotic pressure as the plasma, and the volume which enters each minute is probably about one-sixth of the glomerular filtration rate The output of some constituents of the urine is extremely variable, but there is a fairly rigid upper limit to the rate at which water and sodium salts can be excreted The minute volume in man ranges from a little below 0.5 ml per minute under conditions of dehydration to an upper limit a little above 20 ml. per minute, which is not exceeded even in Diabetes Insipidus. Since the minute volume of *urine never ordinarily exceeds one-sixth of the glomerular* filtration rate, it follows that at least five-sixths of the water of the glomerular filtrate is reabsorbed This has been referred to by Homer Smith as the "Obligatory Phase" in the reabsorption of water It is believed to take place in the proximal convoluted tubule and the loop of Henle, and corresponds to the "bulk-reducing" phase of reabsorption which has been mentioned. Thus normally invariable phase of reabsorption can be [illegible]
doses [illegible]
therefc [illegible]
system is impermeable to glucose in excess of the amount which can be reabsorbed, and to the other three substances named, so that these remain in the tubule where their osmotic activity prevents the reabsorption of water Wesson and Anslow obtained minute volumes up to two-thirds of the rate of glomerular filtration in dogs by the intravenous infusion of concentrated

solutions of mannitol.[105] This would correspond to minute volumes greater than 80 ml. in man. During this spectacular diuresis the osmotic pressure of the urine was approximately equal to that of the plasma, and a considerable fraction of the osmotic pressure of the urine was contributed by mannitol. Since mannitol and sodium salts were the principal solid constituents of an almost isotonic urine, the concentration of sodium in the urine was considerably below its concentration in the plasma; the difference reached as much as 60 meq. per litre in some experiments. The urine contained between 40 and 65% of the water of the glomerular filtrate from which it was formed, but it only contained from 20 to 27% of the filtered sodium. Hence sodium was being reabsorbed; moreover it was being reabsorbed in excess of water, and against a concentration gradient. The water which would normally have been reabsorbed with the sodium remained in the tubular lumen because of the osmotic activity of the mannitol. This dissociation between the reabsorption of sodium and that of water has been taken to indicate that active reabsorption of sodium underlies the obligatory phase of reabsorption of water.[106] The experiment with mannitol demonstrated that the reabsorption of sodium was active by showing that it could still take place against a concentration gradient. If sodium is reabsorbed in the same way under normal conditions, water will leave the tubules passively by osmosis because if it did not do so the fluid in the tubules would be left more dilute than the plasma, and an osmotic gradient would be set up. In the experiments with mannitol the fluid in the tubule *did not become hypotonic when sodium was reabsorbed* because mannitol, which could not be reabsorbed, was left behind and replaced the sodium, so that the reabsorption of water did not keep pace with that of sodium.

If the obligatory reabsorption of water occurs in this way as a passive process secondary to primary transport of sodium by some kind of sodium pump, the fact that water and sodium move

together in this phase of reabsorption is readily explained, for the process is in effect a reabsorption of normal saline. There is thus an obligatory reabsorption of sodium in the proximal tubules, and to signify the change in outlook, Homer Smith now refers to the proximal reabsorption of water as a phase of "passive water reabsorption" to distinguish it from the active reabsorption which concentrates the urine in the distal tubule.

The distal reabsorption of water differs from the proximal phase most obviously in its wide variability under physiological conditions. About 20 ml. of water per minute are left over after the obligatory phase of reabsorption and enter the distal tubule. Since the minute volume varies from about 20 ml to less than 1 ml it follows that up to 19 ml. per minute of the water entering the distal tubule may be reabsorbed, in what is appropriately referred to as a phase of "facultative reabsorption of water" Since the total concentration of the urine varies within wide limits, facultative reabsorption cannot always be isosmotic Water and solutes must be reabsorbed in varied proportions and so, in facultative reabsorption, water and salt do not go together. The reabsorption of water and that of salt are not however completely independent, for if sodium salts, or other solutes, remain unresorbed in the lumen of the tubule, their osmotic activity limits the reabsorption of water. The independent reabsorption of water and of sodium is the means whereby the kidneys regulate the osmotic pressure of the reabsorbed fluid, and so of its "mirror image", the urine, and of all the fluids of the body. The mechanisms which alter the concentration of the urine will now be discussed.

THE ELABORATION OF HYPOTONIC URINE

If one-sixth of the volume of the glomerular filtrate reaches the distal tubule, and this fraction contains all the urea which was present in the original filtrate, then the concentration of urea in the fluid entering the distal tubule will be approximately 6 x 30

mg. per 100 ml, = 180 mg. per 100 ml or 0.03 molar, which is equivalent to 0.03 osmols per litre, because urea is undissociated in solution. Hence if the fluid which enters the distal tubule is isotonic with the plasma, it will have an osmotic pressure corresponding to a total concentration of 0.30 osmols per litre, and urea will be responsible for 10% of this osmotic pressure. In reality some urea will have diffused back to the plasma, but smaller quantities of other organic compounds such as creatinine will be present in addition to urea, and if their contribution to osmotic pressure may be assumed to balance the amount by which the contribution of urea is over-estimated by ignoring its back-diffusion, it will be true to state that about 10% of the osmotic pressure of the fluid entering the distal tubule is due to its content of "Urea etc." Sodium salts contribute the bulk of the remaining 90%. If 20 ml. of this isotonic fluid enter the distal tubule each minute, and if the distal epithelium reabsorbs all the salts but no water and none of the organic constituents, 20 ml. of urine with an osmotic pressure one-tenth that of the plasma will be excreted each minute. This hypotonic urine might be regarded in imagination as a mixture of 2 ml. of an isotonic solution and 18 ml of water. This water which makes the urine hypotonic to the plasma, has been referred to by Homer Smith as "Osmotically free water", made free by the reabsorption of sodium salts in the distal tubule [86] It must be emphasised that this water was brought into the distal tubule by sodium salts which had not been reabsorbed in more proximal parts of the nephron Unless it had thus been brought into the distal tubule it could not have been released there by the reabsorption of sodium, a consideration which may partly explain the failure of a normal water diuresis to occur when the body's stores of sodium are seriously depleted. The facultative reabsorption of sodium which releases osmotically free water in the lumen of the distal tubule depends upon an active transport of sodium through the epithelial cells, and it can proceed against

a greater concentration gradient than the obligatory reabsorption of sodium which leads to isosmotic reabsorption of water in the proximal system, for sodium may at times be almost completely absent from the urine.

The distal tubular epithelium has to perform two feats to produce a dilute urine Not only has it to reabsorb sodium against a considerable concentration gradient but, perhaps even more remarkable, it has to avoid reabsorbing water The back-diffusion of water down its concentration gradient from the low osmotic pressure of the urine to the *higher osmotic* pressure of the plasma has somehow to be prevented. It is a theoretical possibility that water might be secreted outwards into the urine to dilute it, but this is at present an unnecessary hypothesis, for which there is no unequivocal evidence

THE ELABORATION OF HYPERTONIC URINE

When the body runs short of water, the osmotic pressure of the extracellular fluids commences to rise, and Verney has shown how the increase in osmotic pressure causes the neurohypophysis to secrete an antidiuretic hormone Something will be said later about factors which regulate the release of the hormone, but this fascinating chapter of renal physiology should be read in *Verney's own words* [98, 99] When an animal is excreting a concentrated urine, its kidneys are acting under the influence of the antidiuretic hormone secreted by its own pituitary, and the injection of exogenous hormone may have no obvious effect. The typical antidiuretic effect of exogenous hormone is only observed when it is administered to an animal during water diuresis, for then the secretion of endogenous hormone is in suspense.

Two main alterations in function seem to occur when the antidiuretic hormone reaches the kidney during water diuresis.

1. The osmotically free water formed in the distal tubule is reabsorbed This by itself would suffice to reduce the minute

volume and would raise the concentration of the urine to make it isotonic with the plasma.

2. An additional amount of water is reabsorbed by the tubular epithelium actively, against an osmotic gradient, so that the urine becomes hypertonic to the plasma. This second process can proceed in man up to a maximal urinary concentration of 1 4 osmols per litre, a little over four times the concentration of the plasma. Other animals such as dogs and rats can achieve higher concentrations; the record seems to be held by a desert mammal, the kangaroo rat, with a total concentration of 5.7 osmols per litre, almost 20 times that of its plasma.

The first or "Isosmotic-making" phase, the reabsorption of the water which was left behind in the tubule by the reabsorption of sodium salts, might be a phase of passive reabsorption so far as the water is concerned, like the reabsorption of water along with obligatory reabsorption of sodium in the proximal system The real problem which this phase presents is not so much how the antidiuretic hormone causes the reabsorption of the osmotically free water, but how it allows it; and how the reabsorption, or back-diffusion, of this water is prevented in the absence of the hormone. Put crudely, the reabsorption of sodium which leaves the urine hypotonic must take place through a water-proof membrane, and the antidiuretic hormone must reversibly remove the waterproofing. Work done in Copenhagen on a very different organ, the skin of the frog, may provide a clue to this action of the antidiuretic hormone within the kidney.

The permeability of a membrane to water may be determined by measuring the rate at which water flows through it under osmotic or hydrostatic forces. Alternatively, if different isotopic modifications of water are initially present on opposite sides of the membrane, the rates of diffusion of water through the membrane in both directions can be measured simultaneously,

and a net flux calculated which might be expected to agree with the permeability determined by more ordinary methods. But in porous membranes a discrepancy arises between the permeabilities determined by these two methods, because when the fluid moves through the pores in bulk, its flow assists diffusion in the direction of flow and hinders diffusion in the opposite direction. There is thus a net flux by diffusion which becomes added on to the ordinary viscous flow through the membrane, and makes the permeability measured by actual movement of water under osmotic or hydrostatic forces larger than the permeability derived from the net flux measured with isotopes. Koeford-Johnsen and Ussing employed both these methods to study the permeability of frog skin, and found a discrepancy of this kind which indicated that the skin behaved as a porous membrane.[44, 97] They also discovered that when frog skin, mounted between Ringer's solution and a ten-fold dilution of Ringer's solution with water, was treated with posterior pituitary extracts, the osmotic flow and the net flux through the membrane were increased by as much as 100 to 200% but there was no comparable increase in the total flux (The total flux is the sum of the rates of diffusion in the two directions, and is in general much greater than the net flux which is the difference between them.) Now if the pores are large compared with the molecules of the liquid, the filtration, or flow through the membrane under hydrostatic or osmotic forces, is a viscous flow governed by Poiseuille's law, and its rate is therefore proportional to the fourth power of the pore radius. The rate of diffusion on the other hand depends upon the total area of the pores through which it can take place, and hence upon the square of the pore radius. Hence an increase in pore size without alteration in the number of pores should increase the rate of filtration through a porous membrane under osmotic and hydrostatic forces in proportion to r^4, but should only increase the total flux by diffusion in proportion to r^2. On

the basis of such an argument the effect of the antidiuretic hormone has been interpreted as an increase in the pore size of the frog skin, which allows a more rapid equalization of osmotic pressure to take place across it. A similar opening up of pores under the influence of the antidiuretic hormone might account for the reabsorption of osmotically free water in the distal tubule of the kidney.

THE CONCENTRATING MECHANISM

Any attempt to explain the mechanism of the second, or "hyperosmotic-making" phase of facultative water reabsorption must start from the observation that maximal urinary concentrations can only be attained at minimal rates of flow. This fundamental property of the system which concentrates the urine is dramatically demonstrated by the response of a dehydrated man or animal to the intravenous infusion of 10% sodium chloride. When a person has taken no water for 24 hours he produces about 0 5 ml. concentrated urine per minute The infusion of several hundred ml. of 10% sodium chloride, which is extremely hypertonic, raises the osmotic pressure of the extracellular fluids and increases the need to conserve water. The kidneys might be expected to compensate for this, if they were able, by excreting a still smaller volume of more concentrated urine. Instead the minute volume increases enormously, and at the same time the urine becomes more dilute, until its osmotic pressure at the height of the diuresis is little greater than that of the plasma. A normal student, who was producing 0.5 ml. of concentrated urine per minute beforehand, responded to the intravenous infusion of 250 ml. of 10% NaCl with a minute volume of 24 ml, a greater diuresis than could be obtained by drinking water This paradoxical diuretic response when water ought to be conserved more strenuously reveals a limitation which might be expected to throw light upon the way in which the kidney does its work. The problem posed by this diuresis

is of more than academic interest, for sea-water is hypertonic saline, and it is therefore relevant to the controversy about whether survivors of shipwrecks, stranded for an indefinite period on rafts, should be told to drink sea-water or strongly discouraged from doing so

It has been known that the kidney behaves in this way for more than half a century, but it has taken a surprisingly long time to explain so striking a phenomenon. The earliest workers thought that only hypertonic infusions were diuretic, and they noticed that after the intravenous infusion of water the urine became sparse and often bloodstained, a result which would now be attributed to intravascular haemolysis Dreser described diuresis with a fall in urinary concentration following infusion of 10 % NaCl in rabbits in a delightful paper published in 1892. This paper is also notable as one of the first to advocate the use of measurements of the freezing point in place of observations of the haemolysis of erythrocytes for the determination of osmotic pressure in physiological researches [20] As illustrations of the usefulness of this method he noted that the freezing point of his urine on rising in the morning was -2.30° C, but it could be diluted to -0.2°C by drinking beer, whilst the freezing point of his blood serum remained at - 0 56°C ; and he calculated the amount of work his kidneys would have to do to produce these changes in total concentration. He used determinations of the depression of the freezing point to compare the urinary concentrations of rabbits fed on dry and on fresh food, and to show how the concentrated urine of a rabbit fed on dry food increased in volume and became more dilute in response to infusions of 10% NaCl. Galeotti followed this up in 1902 with a more detailed study of dogs in which the same phenomenon was demonstrated in response to infusions of 10% NaCl and of hypertonic solutions of glucose and urea. [30] He also used a variety of drugs to damage the kidneys and concluded that the ability to produce a concentrated urine was not impaired by the

destruction of glomeruli, but depended upon the integrity of the tubules. Starling referred in his book "The Fluids of the Body", published in 1909, to the fact that concentrated infusions produced a copious flow of urine far more dilute than the solution which was infused, and even more dilute than the urine before the infusion. He spoke of the phenomenon as well-known, and gave no references. [91] After that, however, it seemed to be largely forgotten until McCance rediscovered it in man in 1945 and set out the problems which it raised in an important paper which needs to be read more than once. [51]

The following are three factors which might limit the extent to which water can be abstracted from the tubular fluid to make a hypertonic urine

1. A limit to the total concentration in the urine of each individual solute or group of solutes.
2. A limit to the difference in osmotic pressure against which the epithelium can transport water from urine to plasma. This would lead to a limiting total urinary concentration, or "osmotic ceiling", independent of the composition of the urine
3. A limit to the rate at which the tubular epithelium can provide energy to do the work of concentrating the urine This could lead to an impairment of concentrating power at high minute volumes, because less work could be expended per unit volume of urine formed.

The first possibility was suggested by some experiments of Gamble and his associates who fed rats upon mixtures containing high proportions of urea or of various salts, so that either urea or salt could be made to appear at will as the predominant urinary solute [31] The total concentration of a urine which had sodium chloride as its chief solute was found to be 0.6 osmols per litre, whereas urines which contained mainly urea had concentrations up to 1 2 osmols per litre, so that there appeared to be a higher limiting concentration for urea than for salts.

Moreover the two limiting concentrations were to some extent independent because a greater total concentration was attained with suitable mixtures of urea and salt than with either alone; thus urine which contained urea and sodium chloride in the proportion of 2: 1 had a total concentration of 1 6 osmols per litre.

The kidney seemed able to effect a greater economy of water when it was excreting urea than when it was excreting salts, and this was taken to indicate a peculiar fitness of urea to be a metabolic waste product. But in these experiments the rats had free access to water, and although their urine was highly concentrated by human standards, it was more dilute than that of rats deprived of water. These were therefore optimal rather than maximal concentrations and urea might have been excreted with less water than sodium salts because it made the rats less thirsty, and not because their kidneys excreted it by a different mechanism. Nothing in these observations is necessarily inconsistent with the hypothesis that water can be reabsorbed up to a limiting concentration which is independent of the chemical composition of the tubular fluid, because the conditions of the experiments were not such as to test that hypothesis

Hervey, McCance and Tayler [38] published in 1946 an important addendum to McCance's paper of the previous year, in which they reported the results of inducing diuresis in previously dehydrated human subjects in such a way that the osmotic pressure of the urine was mainly due to sucrose, sodium chloride and urea in varying proportions. When diuresis is induced on a background of previous dehydration, it may be assumed that the kidneys are conserving water to the limit of their capacity throughout the experiment, and that the results are not complicated by alterations in the output of antidiuretic hormone from the pituitary. The experiments indicated that the urine could be concentrated up to a limiting osmotic pressure which did not depend upon the nature of the urinary solutes This limiting

osmotic pressure was, however, not constant, but was related in an inverse manner to the minute volume. The osmotic ceiling fell as the output of urine rose, and since sodium chloride increased the output of urine more than did osmotically equivalent amounts of urea, the urea was excreted in a smaller volume of more concentrated urine than the salt. The limiting concentration of a urine containing predominantly urea was greater than that of *a urine containing predominantly salt because its smaller minute* volume allowed it to be more concentrated. Urea cannot be concentrated more than salts at the same minute volume, and so the greater economy of water which Gamble observed is to be explained by the smaller diuretic effect of urea. Hervey et al. ventured the opinion that the depression of the osmotic ceiling might be due to a limit to the rate at which the tubules could perform osmotic work.

The main conclusion that there is an upper limit to the concentration of the urine which is independent of its composition at any minute volume, but which falls as the minute volume increases, was abundantly confirmed three years later by Rapoport and his co-workers, using human subjects who were first prepared by deprivation of water and then received hypertonic infusions of no less than eleven different diuretic solutes [71, 72] (Glucose, urea, creatinine, sorbose, mannitol, p-aminohippurate, sodium chloride, sodium sulphate, xylose, sucrose and sorbitol.) The results of all experiments of this kind fall close to a curve of the general form shown in Fig. 2. A maximal urinary concentration of 1.4 osmols per litre is only reached in man at minute volumes less than 1 ml.; at the height of osmotic diuresis the urine, even of a dehydrated individual, is little more concentrated than the plasma. Rapoport's team also claimed to have established that there was a "biological maximum" of osmotic work beyond which the tubules could provide no more energy to concentrate the urine. But this upper limit of available energy could not account for the dilution of the urine, because it was not reached

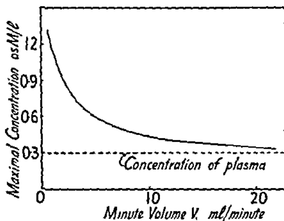


FIGURE 2. Relation of maximal urinary concentration to minute volume.

until the minute volume had been raised to over 10 ml., and the greater part of the fall in urinary concentration occurred while V was increasing from about 0.5 to 10 ml. per minute. Failure of the supply of energy might therefore account for the small depression of the osmotic ceiling as the minute volume increased from 10 to 20 ml., but another explanation must be found for the much greater depression in the interval from $V = 0.5$ to $V = 10$ ml per minute. I have calculated from the published data of Wesson and Anslow the osmotic work performed by the kidneys of their dogs during extreme diuresis induced by infusing hypertonic mannitol solutions, and found that it was increasing more or less in proportion to the minute volume even when this was two-thirds of the glomerular filtration rate; there was no indication whatever that an upper limit might be reached

Calculations of the work done by the kidneys are however so

unsatisfactory that it is doubtful whether they have any value as evidence. The pioneers Dreser and Galeotti both used an over-simple formula which gave the theoretical minimum of free energy required to produce the difference in total concentration between the urine and the plasma According to this formula no work need be performed to elaborate a urine isotonic with the plasma, even though some of its constituents were diluted and others concentrated. An improved formula, which takes into account the work done in altering the concentration of all the constituents of the urine individually was developed by von Rhorer in 1905, and subsequent workers have all employed what is essentially this same formula, though they have arrived at it by different routes of varying elegance.[101] It gives the amount of osmotic work required to form each litre of urine as

$$OW = RT.\ [\Sigma\, c_u .\ \ln \frac{c_u}{c_p} + \Sigma\, c_p - \Sigma\, c_u]., \ldots \quad (6)$$

where R is the gas constant and T the absolute temperature, c_u is the concentration of any substance in the urine and c_p the concentration of the same substance in the plasma. This formula has to be re-expressed in a form suitable for dealing with the experimental results by means of appropriate conversion factors. Calculations made with its aid have agreed in showing that the kidneys perform an amount of work of the order of 1 to 2 Calories per day in their ordinary task of producing the urine During shorter periods of maximal diuresis rates up to 0.25 Calories per hour have been recorded. Compared with the oxygen consumption of the kidneys these figures are small, and suggest that the kidneys work with an overall efficiency of between 1 and 2%. Even this low efficiency is not discreditable however, for the total energy expended (calculated from the renal oxygen consumption) is about as much as could be obtained by burning an ounce of alcohol in a spirit lamp. To produce 1½ litres of urine from 5 litres of blood in 24 hours with the energy

derivable from this small fuel allocation, and to leave the blood fit for use at the end of it all, is a feat which no biochemist has a right to despise.

The efficiency of the kidneys is bound to be underestimated by these calculations, for they can only give the ideal minimum of work required on the assumption that all changes are produced by processes which are reversible in the thermodynamic sense, which means that they must proceed as an infinitely slow succession of equilibrium states. It is also assumed that free energy gained by the dilution of some constituents may be set off against the work required to concentrate others, which is most unlikely to be so in the kidney, especially if different solutes are handled by cells in different parts of the nephrons. Gowland Hopkins once remarked that thermodynamic conditions limit biological possibilities, but they may not be used to predict biological probabilities. Until a new method of calculation is devised they cannot finally answer the question whether the inability of the kidneys to produce a highly concentrated urine copiously is due to a limit to the rate at which the tubular epithelial cells can perform osmotic work or to some other cause. This is not the only explanation possible, and two hitherto unmentioned will now be discussed

Kinetic factors as well as the availability of energy may limit the extent to which a change can be completed Black[6] mentioned this possibility in connection with the excretion of sodium, and the kinetics of diffusion in the tubules might also provide the limiting factor responsible for the depression of the maximal concentration of the urine during osmotic diuresis. If the epithelial cells could abstract water from the fluids in immediate contact with them and maintain a layer with some maximal concentration c_{max} at the periphery of the tubular lumen, then provided that the fluid did not pass down the tubules too rapidly, water would have sufficient time to diffuse from the centre to the more concentrated peripheral layer, and concentration would

become equal to c_{max} right across the lumen. During rapid diuresis the fluid in the axial stream might be hustled out of the concentrating segment before it had come into equilibrium with the peripheral layer, and the subsequent equalization of concentration across the tubule downstream would produce a more dilute urine.

Since this is in some respects an attractive hypothesis the reasons which led to its abandonment may be worth stating. The difficulty of testing it by a quantitative comparison of the rate of flow along the tubule with the rate of diffusion across it was eased by the chance discovery of a formula derived by Grober and Erk for the analogous problem of heat transfer to a fluid flowing along a pipe past a point at which the temperature of the wall abruptly changes from T_0 to T_1.[35] The mean temperature $\overline{T}$ of the fluid at a distance z downstream from the change of wall temperature is given by:—

$$\frac{\overline{T} - T_1}{T_0 - T_1} = 0.819 \exp\left(- 14\ 6272 \frac{k \cdot z}{u_m d^2}\right)$$

$$+ 0.0976 \exp\left(- 89.22 \frac{k \cdot z}{u_m d^2}\right) + \text{smaller terms}, \quad (7)$$

whence $\overline{T} = T_1 + (T_0 - T_1)$.

$$\left[0.819 \exp\left(-14.6272 . \frac{k \cdot z}{u_m d^2}\right) + ..\right] \quad . \ . \ . \ . \ . \quad (8)$$

where u_m = mean velocity of flow,
k = thermometric conductivity of fluid and
d = diameter of the pipe.

Since the equations governing transfer of matter by diffusion are formally similar to those for the conduction of heat, this formula can be adapted to the problem of osmotic diuresis by the following substitutions:—

k is to be replaced by the diffusion coefficient D.

z " " " "	" the length of the distal tubule, l.
d " " " "	" the diameter of the distal tubule, d.
T_0 " " " "	" the concentration of the fluid entering the distal tubule This may be assumed equal to that of the glomerular filtrate, 0 30 osM./l.
T_1 " " " "	" The concentration which is maintained at the periphery This may be taken as equal to the maximum urinary concentration attained at low values of V, say, 1.2 osM /l.

Equation (8) is then replaced by the corresponding expression

$$\bar{c} = 1.2 + (0.3 - 1\,2).\left[0\,819 \exp\left(-14.6272\ \frac{D\,.\,l}{u_m d^2}\right) + \quad ..\right]$$

$$= 1.2 - 0.9\left[0.819 \exp\left(-14\,6272\,\frac{D\,.\,l}{u_m d^2}\right) + \quad \right] \quad \ldots\ldots (9)$$

where $\bar{c}$ is the mean concentration of the fluid emerging from the distal tubule, and d and u_m are now the diameter of the distal tubule and the mean linear velocity of flow along it.

The pure numbers in equation 9 depend upon the use of engineering units, with lengths in metres and times in hours, so that the diffusion coefficient D must be expressed in square metres per hour. Temperatures are dimensionless ratios and do not strictly correspond to concentrations, which have the dimensions of mass divided by volume ($M\,L^{-3}$). The original equation 7 however contains only ratios of temperatures, so that when it is applied to concentrations, the units in which these are expressed are immaterial. Orr and Butler, using deuterium hydroxide, found the coefficient of self-diffusion of water to be $3.88.\ 10^{-5}$ cm^2 per second at 35° C., or say, 4×10^{-5} cm^2/sec. at body

temperature.[63] This corresponds to $4 \times 10^{-9} \times 3{,}600$

$$= 1.5 \times 10^{-5}\ \text{m}^2.\ \text{per hour approximately.}$$

The length of the distal tubule may be taken as 0 01 metre, its *diameter (20μ) as 2×10^{-5} m. If the average flow through the* distal tubules is 20 ml. per minute, distributed evenly over two million nephrons, the flow per nephron will be 10^{-5} ml. per minute. The cross section, πr^2, is approximately 3×10^{-6} cm.^{2}, so that the linear velocity of flow comes to $10^{-5} \div 3 \times 10^{-6}$, or 3 cm. per minute, which means that the time taken to traverse the distal tubule is of the order of 20 seconds. This in itself would practically rule out the kinetics of diffusion as a limiting factor, but it is interesting none-the-less to express the rate of 3 cm. per minute as 2 metres per hour and insert it in equation 9. The result is that the term

$$\frac{D \cdot l}{u_m d^2} = \frac{1.5 \times 10^{-7}}{2 \times 4 \times 10^{-10}} \doteqdot 200.$$

The exponential term is therefore of the order of $0.819\, e^{-2,800}$ which is vanishingly small, and the whole rather fearsome-looking expression reduces itself to $\bar{c} = 1.2$, showing that equilibrium is always reached by diffusion within the lumen, and that the average concentration is the same as the maximal concentration maintained at the periphery. Since this is true for any feasible dimensions or rate of flow, nothing is to be gained by seeking a better expression for u_m which takes account of its variation with the minute volume V. The dimensions of the tubular system are such that diffusion may always be presumed to proceed to equilibrium within the lumen. This calculation is valuable because it completely excludes an explanation which could be made to sound plausible so long as it was not put into quantitative terms. The negative result is a consequence of the minuteness of the distances involved. Matter diffusing in bulk takes a very long time to get far, but it can cover a short distance

surprisingly quickly, because the distance covered is proportional to the square root of the time. This is well illustrated by Table 2 which is taken from a valuable review by Jacobs.[41]

TABLE 2.

Time required to approach within 1% of a steady state of diffusion at distance *H* from the boundary between a solution of fixed concentration and the pure solvent

H	*Time*
10 cm.	52.98 days
1 cm	12.72 hours
1 mm.	7.6 minutes
100 μ	4.56 seconds
10 μ	0.0456 sec.
1 μ	0.000456 sec
0.1 μ	0.00000456 sec

Two rather complicated explanations of the inability of the kidney to fabricate strongly hypertonic urine in large quantities have been discarded; there is another explanation which is so simple that it appears almost trivial, yet which is likely to be correct. This is that there is an upper limit to the rate at which water can be reabsorbed by the segment of nephron which can reabsorb it against a gradient of osmotic pressure, there is likely to be such a limit if this is a relatively short segment. Evidence has recently been obtained[46, 88] that not more than 2 or 3 ml. of *water can be reabsorbed per minute by the human* kidneys against an osmotic pressure gradient. The removal of 3 ml. of water from 4 ml. of *isotonic* fluid would leave 1 ml. of urine with an osmotic pressure four times that of the plasma; but the removal of 3 ml. of water from 23 ml of isotonic tubular fluid would leave 20 ml. of urine with an osmotic pressure 23/20 times that of the plasma. The osmotic pressure would have to fall as the minute volume increased because the small volume of concentrated urine which could be formed by a limited rate

of reabsorption of water unaccompanied by solutes would be swamped in a flood of isotonic fluid. The reabsorption of most of the electrolytes from the fluid entering the distal tubule would leave considerably less than 4 ml. of isotonic fluid per minute for the concentrating segment to operate upon, and the amount of water reabsorbed would then be limited not by the rate of transfer of water across the epithelium of this segment, but by the greatest osmotic gradient which the epithelium could generate; water would continue to be reabsorbed until the upper limit of osmotic pressure was reached in the tubule. Thus there are two distinct limiting factors to the urinary concentration. When the minute volume is low enough, urinary concentration is limited by an osmotic ceiling only. This usually ensures that the solutes which are not reabsorbed from the tubules are accompanied by sufficient water to carry them into the urine in solution, but it may fail to do so when relatively insoluble substances such as acetylated sulphonamides are being excreted. Any freely soluble substance which enters the tubules in the glomerular filtrate and cannot be reabsorbed (such as mannitol), or in larger quantities than can be reabsorbed (such as glucose in diabetes mellitus), or which is excreted by the tubular epithelium and cannot diffuse back (such as p-aminohippurate), will act as an osmotic diuretic. Osmotic diuretics increase the output of urine because they remain in the tubules and increase the volume of fluid left behind after sodium salts have been reabsorbed and enough water has followed them to *leave the tubular fluid isotonic with the plasma.* A second limiting factor determines the concentration of the urine when this residual volume of isotonic tubular fluid exceeds about 4 ml. per minute. Since, even when the antidiuretic hormone is exerting its full influence, the human kidney cannot reabsorb more than 2 or 3 ml. of water per minute against an osmotic gradient, the concentrating process comes to an end before the osmotic ceiling is reached It is important to emphasise that

although the limiting concentration of the urine falls as the minute volume rises, so that the limiting concentration may be conveniently plotted as a function of minute volume, the increase in minute volume must not be regarded as the *cause* of the falling concentration. The greater minute volume and the smaller concentration of the urine are both effects of changes taking place upstream. The greater amount of solute remaining unreabsorbed in the tubule must inevitably be contained in a larger volume of isotonic solution; and the removal from this of the usual 2 or 3 ml. of water per minute leaves a urine which is at the same time both greater in volume and less concentrated. The fact that when more solutes are excreted water ceases to be reabsorbed before the upper limit of osmotic pressure has been reached has the important practical consequence that large doses of osmotic diuretics remove more water from the body than might be expected from their osmotic activity alone, because they remove it in almost isotonic instead of in concentrated urine. Thus a man must lose four times as much water during an osmotic diuresis in which the total concentration of his urine falls to 0.35 osM./l. as he would lose if his kidneys could excrete the same dose of diuretic in a urine concentrated to 1 4 osM./l.

The suggestion that the reabsorption of water against an osmotic gradient is limited to a few ml. per minute has a bearing upon the action of the antidiuretic hormone upon the kidney. It was implied on page 73 that the hormone had two actions, i) it permitted the isosmotic reabsorption of "osmotically free water" left behind by the reabsorption of sodium, and, ii) it switched on the active, "hyper-osmotic-making" phase of water reabsorption. If the segments of distal tubules which carry out this second phase of water reabsorption take up no more than 3 ml. of water per minute even when unopposed by an osmotic gradient, they might be supposed to function continuously, instead of being turned on or off by the presence or absence of

the antidiuretic hormone. In the absence of the hormone they would merely reduce by 3 ml. per minute the rate at which the osmotically free water generated in the diluting segments reached the urine. Hence only a single renal action need be attributed to the antidiuretic hormone, and this is an action analogous to the pore-widening effect which the same hormone exerts upon frog skin. Moreover if the antidiuretic hormone acts only upon the "isosmotic-making" phase of facultative water reabsorption, the well-known fact that this hormone does not inhibit osmotic diuresis is readily explained, for if other solutes which cannot be reabsorbed replace the sodium reabsorbed by the diluting segment, there will be no osmotically free water to return to the blood under the influence of the hormone.

TRANSPORT OF WATER AGAINST OSMOTIC GRADIENTS

It has still not been explained how the tubular epithelium transfers water to the blood from a more concentrated tubular fluid. If the whole gradient is developed across a single layer of epithelium, there may be a difference in concentration of as much as 1 osM./l between the fluids in contact with the opposite poles of the epithelial cells As with the secretion of solutes there are the alternative possibilities of active transport of water across the luminal border or across the basal border of each epithelial cell. If water is transported into the cell at the luminal pole, the osmotic pressure of the cytoplasm will have to be maintained below that of the plasma, in order that water may diffuse out passively across the cell membrane at the basal pole into the peritubular interstitial fluid and blood. On the other hand, active transport of water out of the cells at their basal ends would keep the osmotic pressure of the intracellular fluids higher than that of the general extracellular fluids of the body and would enable water to be absorbed from the tubular lumen passively. The osmotic pressure of the fluid in the lumen could be increased towards an upper limit set by the highest

osmotic pressure that could be maintained inside the cells. The rate at which water could be transported from the lumen to the blood would be limited by the maximal rate of transfer across the cell membrane at the basal pole

One of these alternatives requires the osmotic pressure of the cytoplasm to be less, and the other requires it to be greater than the osmotic pressure of the extracellular fluids, so that either is in conflict with the generally accepted belief that there is never any sustained difference in osmotic pressure between fluids separated by a single thickness of cell membrane. Though both are in a sense equally improbable for this reason, the gathering together of the mitochondria at the basal ends of the epithelial cells might be taken to favour the second alternative, for if the enzymic mechanisms which release energy within cells are for the most part located in the mitochondria, secretory work is likely to be performed where mitochondria are plentiful and arranged in a definite pattern. There is also a certain amount of independent evidence from several sources which, though not compelling, can be interpreted in favour of the hypothesis that the osmotic pressures of the fluids inside the cells of parenchymatous organs, especially the kidney, are significantly greater during life than that of the fluid which surrounds these cells. This evidence has been discussed in some detail elsewhere, and is treated only briefly here. [78, 79]

1. Cryoscopic work carried out half a century ago and several times subsequently confirmed, indicated that the depression of the freezing point of water in the cytoplasm of normal parenchymatous organs was greater than that of the blood by as much as 50 to 100%. These observations might have been vitiated by artefacts in a number of ways, but the differences in osmotic pressure which they appeared to show were abolished in the liver by poisoning with phosphorus, and in all tissues by water intoxication or by dehydration to the point of death.

2. Isolated fragments of organs like liver, kidney and brain have been known since the last century to take up water and swell in solutions having the same osmotic pressure as the fluids which surrounded them in the body. Solutions of approximately the same composition as the extracellular fluids must have about *twice their total concentration* to prevent the swelling of isolated kidney tissue; and there is an interesting old observation that pieces from the medulla require considerably stronger solutions for this purpose than fragments of the cortex. This behaviour is characteristic only of normal organs, and is not shown by those of animals poisoned with such agents as phosphorus or carbon tetrachloride; if these poisoned animals do not die the intracellular hypertonicity which the swelling of their isolated tissues might be taken to indicate returns when they recover
3. Isolated kidney or liver slices which are respiring at body temperature in the flasks of Barcroft manometers do not swell in this way in solutions of the same total concentration as the extracellular fluids. They do however *take up water* and swell to as much as double their initial volume when the gas phase contains no oxygen, when cyanide is added to the medium, or when their metabolism is suppressed by chilling. Moreover the swelling produced by cyanide is reversible; when the cyanide is removed from the medium, the slices expel the extra water which they have taken up. These observations can be interpreted to mean that the volume of the cells is normally restricted, and their cytoplasm maintained hypertonic, as a steady state by active transport of water outwards across the cell membrane fast enough to balance the inward diffusion which must result from the greater internal osmotic pressure. A steady state of this kind would require a continuous supply of energy to maintain it, and it is therefore interesting that 2,4-dinitro-

phenol, in amounts which prevent phosphorylation without much effect upon the rate of oxygen consumption, causes the slices to swell as though poisoned with cyanide.

4. The concentration of total base or of $(Na + K)$ has been found to be greater inside respiring cells than in the medium, in some series of experiments by as much as 50%. Like the power of independent osmoregulation which isolated but surviving cells show, these differences in the concentration of cations depend upon the maintenance of normal respiration and are extremely sensitive to adverse conditions. [1]

Whilst there is no doubt that certain tissue cells in supposedly isotonic surroundings take up water reversibly when their metabolism is impaired, and that their volume depends upon their own metabolism as well as upon external osmotic pressure, it is less certain that this is because intracellular fluids are normally hypertonic. The alternative hypothesis that the amount of osmotically active material inside the cells increases when their respiration is depressed is more generally favoured and has been discussed elsewhere. [76, 78] Some recent cryoscopic measurements made with precautions against known sources of error failed completely to confirm the older work mentioned above. [13] But other workers who also set out to disprove what they regarded as an inherently unlikely hypothesis appear to have made similar measurements with similar precautions but to have obtained results in agreement with the older work. [11] Cryoscopic measurements have not yet been made upon respiring slices which showed high intracellular concentrations of total base on direct analysis, and, until this has been done, the question whether the osmotic pressure of some intracellular fluids is higher than or the same as that of extracellular fluids cannot be regarded as finally settled The fact that the limiting osmolar concentration of the urine does not depend upon the chemical nature of the urinary solutes but only upon their osmotic activity strongly suggests that the urine is made hypertonic by

a process involving active transport of water as such. If it is confirmed that the renal cells have means to maintain an internal osmotic pressure greater than that around them, the same cellular mechanism could account for the production of hypertonic urine. But whether the intracellular fluid is hypertonic or not, it is quite unknown how water molecules are moved across cell membranes against osmotic gradients, although the existence of the urine and of other anisotonic secretions allows no doubt that they are so moved.

THE COUNTER-CURRENT HYPOTHESIS

A mechanism of a completely different nature has recently been proposed to account for the production of hypertonic urine, it has the advantage that no more than a very small osmotic gradient need be assumed across any particular part of the tubular epithelium. Hargitay and Kuhn showed how a small difference in osmotic pressure maintained by some active process (a small head of hydrostatic pressure in their model) could be amplified by a "counter-current diffusion system". The properties of this system were developed mathematically.[37] It operates approximately in the following manner, illustrated in Fig. 3. Two long, narrow tubes A and B (Fig. 3*a*) share a common longitudinal wall of semipermeable material and may be connected at their lower ends by opening a tap *T*. Suppose that both tubes contain initially a solution of osmotic pressure P, and let a hydrostatic pressure $2p$ (small compared with P) be applied to the fluid in the right hand tube, B Water will pass from right to left through the membrane, diluting the fluid in A and concentrating the fluid in B until the osmotic pressures are $P + p$ on the right hand side, and $P - p$ on the left. (Fig. 3*b*) The difference in osmotic pressure will then be $2p$ and this will balance the applied hydrostatic pressure over the whole of the membrane. Now let the tap be opened to allow one half of the fluid in B to flow into A. The fluid on both sides of the lower

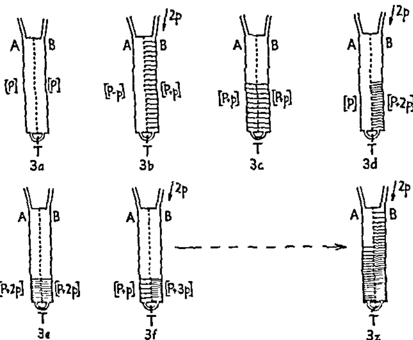


FIGURE 3. To illustrate the countercurrent hypothesis. Explanation in text.

half of the membrane will now have the same osmotic pressure, $P + p$, (Fig. 3c) and if the same hydrostatic pressure continues to act, water will again pass across the membrane from right to left, until the osmotic pressure is $P + 2p$ in the lower half of tube B, and P in the lower half of the tube A. (Fig. 3d). If the tap is again opened until one quarter of the fluid in B has passed into A, the fluid on both sides of the lower quarter of the membrane will once more have the same osmotic pressure, time $P + 2p$. (Fig. 3e). If the hydrostatic pressure $2p$

water will again traverse the lower quarter of the membrane *from right to left until the concentrations in the lower quarters* of the two tubes have become $P + 3p$ and $P + p$. (Fig. 3*f*). And so on . . . . . If instead of conducting this imaginary experiment in discrete steps, the tap T is left slightly open all the time, so that there is a slow flow of fluid through the system, down tube B and up tube A, whilst the head of pressure $2p$ is maintained between the two sides of the membrane, the apparatus will operate continuously until a steady state is finally set up in which the solute is concentrated towards the lower ends of the tubes, and the difference in hydrostatic pressure is balanced against a corresponding difference in osmotic pressure over the whole length of the membrane. The difference in osmotic pressure between the top and bottom of the tubes may be much larger than the difference $2p$ between the two sides of the membrane at any level, but will be limited by the tendency of the concentration to disperse by diffusion. This final steady state is depicted in Fig. 3*z*

The essential feature of this system is a long tube which makes a hairpin bend and doubles back upon itself with the two limbs in contact through a membrane across which water can be transported by some means against a small osmotic gradient represented by $2p$ The currents of fluid in the two limbs of the hairpin flow in opposite directions, hence the picturesque name "hairpin countercurrent system". The continuous operation of such a system leads to a stratification of osmotic pressure in the long axis of the hairpin, such that the difference in osmotic pressure between the fluid entering and that leaving the whole system is no more than $2p$, but the difference in osmotic pressure between either of these and the fluid near the bend is greater. The theoretical calculations were checked by constructing a model apparatus, which behaved in accordance with expectations.

Wirz, Hargitay and Kuhn devised a micro-cryoscopic tech-

nique which depended upon the use of a polarizing microscope to detect tiny crystals of ice formed during the cooling of sections of tissue 30 μ in thickness.[111] They employed this technique to determine the osmotic pressure of the fluid in transverse frozen sections of the kidneys of rats, cut perpendicular to the axis of the renal papilla, and they obtained results which were remarkable in two respects First, the fluids in all parts of the renal tubule at any given depth below the cortical surface of the kidney had the same osmotic pressure. But, secondly, although the fluid in all cross-sections of the nephron in the outer parts of the cortex was isotonic with the plasma, the osmotic pressure of the fluid in all cross-sections increased steadily with the depth below the surface of the kidney to reach values deep in the medulla as much as four times the osmotic pressure of the plasma in the blood vessels. This stratification of osmotic pressures recalls the similar distribution of chloride which Ljungberg found in the rabbit's kidney.[48] The outermost parts of the cortex contained about the same concentration of chloride as the plasma, but there was a steady increase towards three or four times that concentration in the medulla. The higher concentration of chloride at increasing depths below the surface was not found in the kidneys of animals which had previously received a non-lethal dose of cyanide.

Such an increase in concentration of the fluid in the inner parts of the kidney might have been produced by transport of water through cells in the manner suggested in the previous section, but the deeper parts of the medulla, towards the apex of the renal papilla where osmotic pressure was greatest, contain only loops of Henle, collecting ducts and vasa recta, the typically "secretory" parts of the nephron are absent. On the other hand the stratification of osmotic pressure which has now been demonstrated was just what might be expected if the kidney contained a battery of hairpin countercurrent diffusion systems arranged parallel to the axis of the renal papilla. Since the loops

of Henle had precisely this arrangement and possessed hairpin bends, and since no certain function had ever been found for them, it was tempting to suggest that they somehow acted as counter-current systems. The association between the presence of loops of Henle and the ability to secrete hypertonic urine was thus given a new significance. It was suggested that the small unit difference of osmotic pressure ($2p$ in the model, Fig. 3) might be provided by transport of water from the descending to the ascending limb at each level by electro-osmosis. The stratification established when this small difference had been amplified by the counter-current arrangement could then be utilized to concentrate the urine. The fluid in the proximal tubule, being in the cortex, would be isotonic with the plasma. On its way down the descending limb of the loop of Henle towards the medulla it would become progressively more concentrated as it came into equilibrium with the local osmotic pressure prevailing in each layer through which it passed. It would be maximally concentrated at the lowermost point of the loop, but once round the bend it would become diluted again as its journey through the ascending limb brought it back towards the cortex The fluid would be isotonic with the plasma in the distal tubule, which also lies in the cortex, but as it left the distal tubule, it would be carried down through the medulla a second time in the collecting duct, becoming more concentrated on the way; and this time it would not return, but would continue into the bladder as hypertonic urine. The abstraction of water from the tubular urine to concentrate it would therefore be the last operation to be performed upon it, but would be performed beyond the confines of the nephron proper, in the collecting ducts, and moreover by a passive process of reabsorption of water by osmosis.

The golden hamster possesses a long thin renal papilla which projects into the ureter, and Wirz has recently succeeded in obtaining blood from capillaries near the apex of this papilla by

means of a micropipette while the kidney was secreting a hypertonic urine.[110] He found that blood from vessels in the papilla had the same osmotic pressure as the urine. This suggested that in the living kidney, the stratification of osmotic pressures found in the dead organ affects the blood in the vessels as well as the fluid in the nephrons, and it appeared to provide a crucial test in favour of the new conception of the concentrating mechanism; for if the urine had been concentrated by active transport of water from tubular lumen to blood, the blood should have been diluted.

This revolutionary theory of renal function is at variance with the accepted idea that the osmotic pressure remains unchanged until the tubular fluid enters the distal tubule, and it postulates that the tubular fluid is always isotonic with the plasma in its immediate neighbourhood. It conjures up a vision of red blood corpuscles opening and closing like concertinas as the blood flows through the kidneys, and it is a somewhat roundabout method of concentration, though no more roundabout than the sequence of filtration and reabsorption as a method of excretion. Its great advantage is that it does away with the need for cells to secrete water as such against large osmotic gradients. It suggests a function for the loops of Henle, but at the same time may impose upon them the requirement of a more pronounced selective permeability than the customary views demand, for the loops must prevent the back-diffusion of the organic constituents of the urine present in high, instead of only in low concentrations. It also suggests that a special physiological significance might be found for the circulation through the juxtamedullary glomeruli and the vasa recta which lie among the longer loops of Henle of the juxtamedullary nephrons. It is doubtful however whether the two limbs of the loops of Henle can be thought of as the parallel tubes of the counter-current arrangement, for they must be separated by vasa recta more often than not. Moreover since the vasa recta also descend to varying

depths in the medulla, and then turn back towards the cortex at hairpin bends of their own, the blood in any loop chosen at random may be streaming in either direction, and a rather definite anatomical relation of descending limbs to vasa recta running towards the cortex would be required for the system to operate by transport of water from the fluid in the loops to the blood in the vasa recta This however is a question of detail which future work may answer.

The proposed mechanism would be convenient and economical for an animal which habitually produced a concentrated urine. For man, who drinks for other reasons than thirst, and relies upon his kidneys to reject the excess, it would seem less well suited. It would presumably take some considerable time to establish the stratification of osmotic pressure within the kidney upon which this method of concentrating the urine depends; and some time also to dissipate it during water diuresis, and to re-establish it in response to an injection of antidiuretic hormone. Whether or not this theory proves acceptable may depend upon how well it can account for the rapidity with which the kidney can alter the concentration of the urine. Although the authors suggested that the antidiuretic hormone might determine the direction of electro-osmosis by influencing the electrical properties of the membrane, it is not certain whether this mechanism could be adapted to dilute the urine, and there do not seem to be any observations of the distribution of osmotic pressure in the blood and the tubular fluids of kidneys excreting hypotonic urine. It also seems possible that a similar stratification of osmotic pressure might arise in another way in the kidney of an animal whose urine had been hypertonic for many hours. At increasing depths below the surface of the kidney, as the apex of the renal papilla is approached, more and more loops of Henle and vasa recta have turned back towards the cortex, but the collecting ducts continue, and form an ever-increasing proportion of the dwindling cross-section of the

pyramid. The permeability of the walls of the collecting ducts to water is presumably low, but may not be negligible. Over a period of many hours the interstitial fluid around them, and the blood in the relatively small number of vessels which accompany them, might be expected to come almost if not quite into osmotic equilibrium with the concentrated fluid within the collecting ducts. Kinetic experiments, by showing how quickly the stratification could arise, and in particular, whether it appeared before the urine became concentrated, or not until some time later, would help to decide whether the stratification of osmotic pressure within the kidney is the means whereby the urine is concentrated, or merely a consequence of prolonged concentration by other means

V. RENAL CONTROL OF ACID BASE BALANCE.

The maintenance of acid base balance within the organism presents a perpetual problem because the tissues, of which those of the central nervous system are most exacting, cannot function normally unless the pH of the plasma is kept close to 7.4; and this has to be done in spite of the continual generation of acids by metabolism. The bulk of the acid produced in this way is carbonic acid formed from carbon dioxide which is a by-product of the oxidation of foodstuffs. The amount formed each day by an adult man is about 15,000 meq., equivalent to 15 litres of normal acid, or a litre and a half of concentrated hydrochloric acid. Carbon dioxide, the anhydride of carbonic acid, is volatile, and can be eliminated from the body by the lungs; and the respiratory centre is so organized that an increase in the concentration of carbon dioxide in the body fluids increases the rate of pulmonary ventilation. Besides this volatile acid, between 50 and 100 meq. of fixed acids are produced each day in excess of the fixed base taken into the body in food, and these fixed acids have to be eliminated by the kidneys. They include sulphuric acid formed in the degradation of sulphur-containing amino acids, and phosphoric acid from phosphoproteins and phospholipids. In the glomerular filtrate the anions, or acid radicals, of these fixed acids are balanced by their equivalent of the fixed bases of the body, principally by sodium. As has already been stated, the osmotic pressure and the volume of the extracellular fluids depend to a great extent upon the quantity of sodium which they contain, and since this is normally no more than about 1,600 meq., it follows that to allow between 50 and 100 meq. of sodium to escape each day with the excess

of fixed acids in the urine would lead to a rapid loss of the extracellular fluids.

The problem facing the kidneys is to excrete the fixed acids without losing the fixed base which accompanied them into the glomerular filtrate This problem is solved by providing other cations to balance the anions of the fixed acids in the urine, so that the essential fixed base may be reabsorbed and restored to the plasma. Two cations are employed for this purpose, hydrogen and ammonium, and the evidence is that both are secreted by the distal tubules

Replacement by hydrogen ions means that the acid radicals are excreted as free acids, and there is a limit to the amount of acid which can be excreted in this form, because the kidneys cannot secrete urine which is more acid than about pH 4.6. The concentration of hydrogen ions to which a strong acid gives rise in solution may be reduced by dilution with water, but not to any useful extent, because 100 meq of strong acid would have to be diluted to 10,000 litres to raise its pH to 5.0. Hence even in the most acid urine that the kidneys can elaborate, all strong acids must be accompanied by their full equivalent of base. Weaker acids can be excreted in the free form to an extent which is determined by their pK values If pK is the same as the pH of the urine, one half of the acid will be present in the free form. If pK is one pH unit above the pH of the urine, nine-tenths of the acid will be free and one-tenth combined with base. Only one-tenth of the total amount of an acid whose pK is one pH unit below the pH of the urine can be excreted in the free form, so that nine-tenths of its equivalent of base will have to accompany it, and stronger acids, with pK values more than one unit below the minimal urinary pH, must always be excreted with more than nine-tenths of their equivalent of base. This is all summed up in the Henderson-Hasselbalch equation:—

$$pH = pK + \log \frac{\text{Concentration of salt}}{\text{Concentration of free acid}}.$$

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These are important limitations because, as Gamble and Pitts have pointed out, they depend only upon the lower limit of urinary pH and the physical properties of individual acids, and not upon the mechanism by which the urine is acidified. They are therefore of permanent validity whatever theory of acid secretion may be fashionable.[32, 67]

Those weaker acids which can be excreted to a considerable extent in the free form in urine within the physiological range of pH are known collectively as the buffer acids of the urine. The most important of them is phosphoric acid, but amino-acids and creatinine (pK 4.97) are also included in this category. pK for the dissociation of the second hydrogen ion of phosphoric acid is 6.8, so that each equivalent of phosphate in the urine at pH 4 8 requires one equivalent of base to "cover" it, in Gamble's expressive phrase, whereas in the plasma at pH 7.4 it required *nearly two equivalents. Hence by excreting urine of maximal* acidity the kidneys can salvage an equivalent of fixed base for each equivalent of acid phosphate in the urine, as well as additional amounts corresponding to the other buffer acids which are present. The process of acidification is reversed *in vitro* when the urine is titrated with alkali back to the pH of the plasma (7.4) to determine its "titratable acidity". Hence the amount of alkali added during the titration of any volume of urine is equal to the amount of base which the kidneys conserved by acidifying it; and the titratable acidity in meq. per litre, multiplied by the daily output of urine in litres, gives the amount of base conserved by this mechanism during each 24 hours. In a healthy man this amounts to between 20 and 30 meq., but it may increase to as much as 150 meq. during diabetic ketosis, when the urine contains large quantities of β-hydroxybutyric acid (pK 4.7), and aceto-acetic acid.

Although strong acids must be accompanied by their full equivalent of base in *urine of any pH, this need not all* be fixed base. Ammonium can be formed from ammonia synthesized

in the cells of the tubular epithelium, and since this cation is not present in the glomerular filtrate, an amount of fixed base equivalent to the ammonium in the urine can be reabsorbed. Normal human urine contains between 30 and 50 meq. of ammonium each day, and the amount may increase to as much as 500 meq. under the stress of diabetic acidosis. It may be noted that the daily excess of metabolic fixed acid is covered by the sum of the free acid and the ammonium in normal urine, and can therefore all be eliminated without fixed base being lost from the body In severe diabetic acidosis the kidneys are less successful in maintaining acid base balance, for the total amount of metabolic acids which have to be excreted may exceed the amounts of hydrogen and ammonium ions that the kidneys can provide The balance of metabolic acid is then inevitably excreted in combination with fixed base from the body, and this contributes to the gross dehydration and circulatory collapse which threaten the life of the patient But always, whether acid base balance is completely maintained or not, the sum of the titratable acid and the ammonium of the urine is equal to the amount of fixed base conserved by the kidneys.

THE EXCRETION OF ALKALINE URINES

Unlike the secretion of acid urine, which always helps to conserve the body's stores of fixed base, alkaline urines cannot be excreted without the loss of fixed base from the body. There is an upper limit to the alkalinity of the urine at about pH 8, and excess cations in alkaline urine are balanced by bicarbonate, much as excess anions in acid urine are balanced by ammonium. The former is the anion of a volatile acid, and the latter the cation of a volatile base Bicarbonate ordinarily appears in the urine in large amounts when its concentration in the plasma exceeds the normal value of about 27 meq per litre; when the concentration in the plasma falls below this, only traces of bicarbonate are to be found in the urine, yet there is no true

"bicarbonate threshold", for the statement that bicarbonate is practically absent from the urine at plasma concentrations below 27 meq. per litre is only true so long as the respiratory centre is free to regulate *pulmonary ventilation in response to the* prevailing level of plasma carbon dioxide. During a period of voluntary or hysterical over-ventilation the urine may be alkaline and contain considerable amounts of bicarbonate, although the plasma bicarbonate is well below the normal "threshold" concentration. This appears less paradoxical if the kidneys are regarded as regulating, not the level of bicarbonate in the plasma, but the concentration of fixed base. In the words of Pitts, "*the kidney stabilizes the concentration of base in the* body fluids at a level some 25 to 27 meq./l. above that of the sum of all the fixed, non-volatile anions" [68]. Carbon dioxide is always being generated in the cells, and is a ready source of carbonic acid, and so of bicarbonate ions, which are available as a make-weight to ensure that the sum of the anions in all the body fluids is always equal to the sum of the cations. Over-breathing removes carbon dioxide from the body and makes *the plasma alkaline, so that more cations are balanced in the* plasma by the anionic groups of plasma proteins. Since the latter do not reach the glomerular filtrate in appreciable concentration, those cations, principally sodium, which were balanced by protein anions in the plasma, acquire bicarbonate ions as partners in the tubular fluid, for the cells lining the tubules not only produce carbon dioxide, but they also contain the enzyme carbonic anhydrase to hasten its hydration to carbonic acid. *If these cations are not reabsorbed, they finally appear as bicar-*bonate in an alkaline urine. Meanwhile their osmotic activity in the tubules leads to the familiar increase in minute volume which accompanies the increase in urinary pH during hyperventilation. Bicarbonate is excreted in the alkaline urine although the concentration of bicarbonate in the plasma is low, but the bicarbonate of the urine need not have come from the depleted

plasma; it may have come instead from the kidney, where it was synthesized in the epithelial cells and captured by the alkaline fluid flowing past them

The essential action of the kidney in excreting an alkaline urine is to refrain from reabsorbing fixed base, whilst at the same time reabsorption of fixed anions continues or is enhanced. When a person over-breathes during a severe deficiency of sodium there is no diuresis and the urine does not become alkaline, as McCance and Widdowson have shown.[65] The kidney continues to reabsorb sodium actively, and it cannot make the urine alkaline Patients who lose large amounts of sodium from their bodies, and who also develop alkalosis, a combination which may arise from prolonged vomiting or from the aspiration of gastric secretions, may pass acid urine and be in danger of having their alkalosis mistaken for acidosis.[50] If they are supplied with sodium chloride, which is neutral but is a source of sodium, their urine becomes alkaline Alkalosis superimposed upon a deficiency of sodium leads to a conflict between two regulatory functions of the kidney, and the regulation of acid-base balance is sacrificed to the conservation of the body's stores of fixed base. The fact that alkaline urines cannot be secreted without the loss of fixed base from the body is of no consequence to people living on vegetarian diets, for these supply an excess of fixed base (in this case mainly potassium) over metabolic fixed acids. But it is fortunate for people who live on ordinary diets that alkalosis is rather an uncommon event Human urine is usually slightly acid, with a pH around 6.0. This is close to the pK of the bicarbonate buffer system, and on the acid side of the pK of the phosphate system Hence the capacity of the phosphate buffers in the urine is practically used up in combating the normal daily excess of metabolic acid, leaving the ammonia mechanism in reserve to cope with a further degree of acidosis and the bicarbonate system in reserve to cope with the rarer contingency of alkalosis.

THE EXCRETION OF ACID AND AMMONIA

The acidification of the urine appears to depend upon direct secretion of hydrogen ions into the lumen of the distal tubule. The ratio of the concentrations of hydrogen ions in a urine of pH 4.6 and in plasma at pH 7.4 is of the order of 600 to 1, a higher ratio than the kidney can achieve for most substances. This alone might suggest that the primary process was an active secretion of hydrogen ions. Two other theories were formerly held, the phosphate reabsorption theory according to which the tubules reabsorbed the Na_2HPO_4 and left behind the NaH_2PO_4 from the mixture of both phosphates in the glomerular filtrate; and *the carbonic acid filtration theory*, which supposed that by active reabsorption of the bicarbonate ions released by ionisation of carbonic acid, all the phosphate of the glomerular filtrate could be converted into acid phosphate. The former theory could account for secretion of titratable acid up to the rate of glomerular filtration of acid phosphate, and the latter up to the combined rates of filtration of acid and alkaline phosphates. But work in Pitts' laboratory showed that neither theory could account for the rates of secretion actually observed, and they were accordingly displaced by the theory of secretion of hydrogen ions up to a limiting concentration ratio indicated by *the lower limit of urinary pH. On this theory the total* capacity of the urinary buffers determines the amount of hydrogen ions which can be secreted, for it is the amount required to titrate these buffers to the pH of the urine. The hydrogen ions which are secreted into the tubules are believed to be derived from carbonic acid formed from carbon dioxide released by metabolism within the epithelial cells, for Pitts and his colleagues also showed that inhibitors of carbonic anhydrase prevented the secretion of acid, and caused the urine to become alkaline even during an experimentally induced acidosis. This effect of inhibitors of carbonic anhydrase, which are sulphonamide

derivatives, recalls the acidosis associated with an alkaline urine which was a complication of therapy with sulphonamides in the days of their early triumphs. The situation here is not simply that the kidney is paradoxically excreting alkaline urine during a state of systemic acidosis; the kidney produces the alkaline urine because it has been deprived of the wherewithal to excrete acid. The systemic acidosis arises because the plasma takes up an acid reabsorbed fluid, of which the alkaline urine is the "*mirror-image*". *The employment of the same metabolic end-product*, carbon dioxide, as a common source of bicarbonate ions to make the urine alkaline and of hydrogen ions to make it acid is an interesting example of physiological economy.

The ammonium ions in the urine are derived from ammonia produced in the kidney from two principal sources, the glutamine of the blood, which is the major source, and certain α-amino acids. The kidneys contain the enzyme glutaminase which splits off ammonia from the amide group of glutamine, and also α-amino acid oxidases which release ammonia in the course of oxidative deamination. Glutaminase is most active *in vitro* on the alkaline side of neutrality at an optimal pH of about 8.0, but the physiological secretion of ammonia does not reflect the sensitivity of the isolated enzyme to pH Little ammonium is excreted in urines which are not distinctly acid, substantial amounts do not appear until the pH of the urine falls below 6.0. Moreover the rate of excretion of ammonium — not its concentration in the urine — increases in an almost linear manner as the pH of the urine falls,[21] which might suggest *that the acidity of the fluid in the tubules was the stimulus* required to elicit a secretion of ammonia from the epithelial cells. This suggestion is supported by the demonstration that surviving slices cut from the kidneys of rats, and respiring in Barcroft manometers, formed ammonia at a rate which depended upon the reaction of their medium in the same way as the rate of excretion of ammonium by intact rats depended upon the pH

of their urine.[77] The absolute rates at which the slices and the intact kidneys formed ammonia were about the same when expressed per unit weight of renal cortical tissue. It cannot be supposed that nervous or humoral mechanisms which might conceivably regulate the secretion of ammonia by the renal tubules *in vivo* played any part in controlling the formation of ammonia under the standardized conditions of manometric experiments. Consideration of the manner in which external acidity might stimulate the cells to produce ammonia suggests that this might be regarded not merely as a stimulus, but more directly as a cause. If the reactions whereby ammonia is generated in the epithelium are reversible, its concentration inside the cells will be stabilized by a dynamic equilibrium between consumption and production. Ammonia will have little tendency to diffuse out of the cells into an alkaline medium, but an acid medium can fix it by conversion into ammonium ions, and so maintain a low concentration of free ammonia on the outer side of the cell membrane. Diffusion of ammonia out of the cells into the acid medium would lower its concentration within them, and accelerate the reactions producing it. Only if the ammonia-producing enzymes were saturated should their activity, and the effect of pH upon it, become factors limiting the rate of ammonia formation. There is thus no necessary discrepancy between the known pH optimum of glutaminase and the suggestion that the pH outside the cells determines the rate of secretion of ammonium. The much smaller escape of ammonia from the tubular epithelium into the blood, compared with its rate of excretion in the urine, is explained by the higher pH of the plasma. Moreover, if the pH of the intracellular fluids is lower than that of the plasma, and there are grounds for supposing that it may be as much as 1 pH unit lower, the fact that not much ammonium is excreted until the pH of the urine falls below 6.0 is explained.

The two most important consequences of this conception of

the secretion of ammonium by the kidney remain to be mentioned. The first is that it is not a process of secretion at all if that term is used to denote active transport; ammonia leaves the cells spontaneously by diffusion down a concentration gradient, and no energy need be expended by the cells to expel it The second *is that if the ammonia diffuses passively into an acid fluid,* where it is fixed as ammonium, that fluid has first to be made acid by prior secretion of the hydrogen ions which later *combine with ammonia to form ammonium ions* Since acid has to be secreted before it can be neutralised by ammonia, it follows that the amount of hydrogen ions secreted is not merely *equal to the titratable acid of the urine, but to the sum of the* titratable acid and the ammonium, and therefore to the whole amount of fixed base conserved by the kidneys.

It is a further consequence of the hypothesis that the "secretion" of ammonium is secondary to a true secretion of hydrogen ions, that what has been interpreted as an increase in the efficiency of ammonia secretion during a prolonged acidosis might be due to an enhanced secretion of acid. It has sometimes been found after several days of acidosis that urine of a given pH contains more ammonium than urine of the same pH on an earlier day. The larger output of ammonium might be the result of an adaptive increase in the activity of the glutaminase in the kidney, and such an adaptive increase has been demonstrated in rats, but only when the acidosis was continued for periods *of several months.* [14] The increase in ammonium output from day to day may be too rapid to attribute to an increase in glutaminase activity, and it could be accounted for by a greater *intensity of acid secretion. For a tubular fluid which was initially* more acid would require more ammonia to neutralise it, the urine formed from it would have to contain more ammonium than urine of the same pH formed by neutralisation of an initially less acid tubular fluid.

THE MECHANISM OF HYDROGEN ION SECRETION

The most important conclusion reached in the preceding section was that the quantity of hydrogen ions secreted by the kidney is exactly equivalent to the amount of base conserved for the body. This might seem to be an additional argument in favour of the thesis that the urine is made acid by active secretion of hydrogen ions; but the question arises, which of the two processes, secretion of acid and reabsorption of base, represents the cart, and which the horse? Does the secretion of hydrogen ions allow base to be reabsorbed, or might not the primary process be an active reabsorption of cations through a membrane which restricts the movement of the anions? For this could lead to the "secretion" of hydrogen ions as passively as the acidity of the tubular fluid leads to "secretion" of ammonia, and alkalinity to "secretion" of bicarbonate.

If sodium ions are reabsorbed in excess of anions, other cations, equivalent in quantity to the anions left behind, must leave the cells and move in the opposite direction to preserve electrical neutrality in the lumen. Of the ions which are available hydrogen might not at first sight appear suitable for this exchange, because of its extremely small concentration in intracellular fluids. It possesses however the greatest mobility of all ions, and its small intracellular stores could be replenished from carbonic acid formed rapidly by carbonic anhydrase from carbon dioxide released in the cells by their respiration. The generation of hydrogen ions in this way would at the same time free an equivalent quantity of bicarbonate ions to accompany reabsorbed base into the plasma If hydrogen ions were not made available for exchange with sodium reabsorbed from the lumen, potassium might be expected to leave the cells instead, for it is the most abundant cation in the intracellular fluid. It is therefore of great interest that inhibitors of carbonic anhydrase administered to dogs during acidosis not only prevented the acidification of the urine and greatly increased the rate of excre-

tion of sodium *in it*, but also led to the excretion of potassium with a clearance greater than that of inulin This was naturally interpreted as an active tubular excretion of potassium [3,4] Secretion of potassium ions appeared to have replaced secretion of hydrogen ions, and it was suggested that the two cations were competitors for a common channel or mechanism of excretion But although far more of the sodium of the glomerular filtrate was appearing in the urine, the reabsorption had not been completely stopped by the inhibition of carbonic anhydrase, and as reabsorption of sodium was still continuing, conditions tending to withdraw other cations from the cells were still present. When mercurial diuretics were administered to acidotic dogs after the renal carbonic anhydrase had been inhibited, the rate of excretion of sodium in the urine increased further, indicating that the reabsorption of sodium was now more completely inhibited, and with this further inhibition of the reabsorption of sodium the tubular excretion of potassium ceased.

Instead therefore of regarding hydrogen and potassium as competitors for excretion in the urine, it might be more profitable to regard the acidification of the urine as a normal physiological consequence of active reabsorption of sodium, and the excretion of potassium from the tubular epithelium as an abnormal consequence, which occurs under relatively unphysiological conditions when hydrogen ions are not freely available to replace sodium reabsorbed from the tubular fluid. Looked at in this way the tubular "secretion" of potassium may be passive in two senses, both as being secondary to a primary active transport of sodium, and probably also as being in the direction of its concentration gradient from intracellular fluid to tubular urine It should however be emphasised that even if the movement of potassium from tubular epithelium to tubular fluid is passive, the high intracellular concentration of potassium that makes it so may still depend upon an active transfer of potassium from blood to tubular epithelium at the opposite poles of th

cells. Whether this inward movement of potassium could also be secondary to an active extrusion of sodium through the basal ends of the cells with their closely packed mitochondria must remain for the future to decide.

Sodium pumps have been invoked to account for the obligatory phase of reabsorption of sodium and water in the proximal parts of the nephrons, and sodium transport through a segment of epithelium with a low permeability to water has been suggested as the mechanism whereby hypotonic urine is formed. If the active transport of sodium through a third segment of epithelium which restricts the movement of anions can account for the secretion of acid, and so secondarily of ammonium, and sometimes of potassium, it becomes clear that the transport of sodium plays a remarkably large part in the operations of the kidney. This is a satisfying conclusion, for active transport of sodium is an almost universal property of mammalian cells, most of which exist in sodium-rich extracellular fluids and yet contain little sodium within them. Whatever the mechanism of the sodium pumps, they presumably operate outwards around most of the periphery of most cells. Besides their part in maintaining the characteristic difference in the ionic make-up of intracellular and extracellular fluids, they subserve other functions in specialised cells, as for instance in nerve axons, where the uneven distribution of ions maintained across the membrane allows impulses to be conducted along it. In the renal tubular epithelium, sodium must be actively transported outwards by a mechanism which is located predominantly at one end of the cells, so that instead of merely extruding sodium round the periphery to maintain a low intracellular concentration without much movement through the cells, the pumps move a current of sodium and water through the cells at a considerable rate. The glomerular filtrate formed each minute totals about one-half of the weight of tubular epithelium, and most of this filtrate is reabsorbed.

If the urinary excretion of acid and of ammonium can be explained by a mechanism which is common to most cells and the probable basis of two other major renal operations, the workings of the kidney can be described in a greatly simplified form Moreover the old statement that the amount of base *conserved by the kidneys is equal to the sum of the ammonium* and the titratable acid which they excrete, acquires a new and a fuller significance when conservation of base is regarded as the primary, active process, and the excretion of acid and ammonia as *secondary*. *But it must be admitted that the interpretation of* the secretion of acid as a consequence of the active reabsorption of sodium may turn out to be more of a mnemonic device than a true explanation, for it is not known how cells transport sodium across their membranes According to Davies and Ogston[37] sodium is not an ion which can undergo primary secretion, like hydrogen which can be generated by metabolic oxidation from unionized precursors, so that unless a suitable carrier can be found to transport sodium in an unionized form, the transport of sodium ions might in the end have to be interpreted as secondary to that of hydrogen ions, but that is a problem *for* the future, and *for* general physiologists and biochemists.

If enough were known about it, this chapter might end with an account of the regulation of the kidneys' operations in defence of acid base balance. There is no convincing evidence that nervous mechanisms play a part in regulating the excretion of acids and alkalis by the kidneys The pH of the plasma might determine the response of the kidney, for the reaction of the glomerular filtrate presumably reflects that of the plasma. Moreover changes in the pH of the plasma are partly buffered by the plasma proteins, and since no substantial concentrations of these reach the glomerular filtrate, changes of pH in the filtrate may be greater than those in the plasma If tubular reabsorption remained constant, the relative excess or deficit

of fixed cations associated with high or low pH of the plasma would be further amplified by the tubules. But recent work seems to indicate that the reabsorption of "bicarbonate-bound base" (fixed cations unaccompanied by fixed anions) varies with the partial pressure of carbon dioxide in the plasma irrespective of pH.[69, 74] This effect of carbon dioxide in promoting the reabsorption of base may assist the kidneys to compensate for the "respiratory acidosis" which accompanies retention of carbon dioxide in the body, and to excrete alkaline urine during hyperventilation.

If, as has been suggested, the secretion of ammonium and of acid are in turn consequences of reabsorption of sodium, then the regulation of these functions of the kidney might be effected by those hormones of the suprarenal cortex which are known to promote the reabsorption of sodium by the tubules. Since the excretion of acid and ammonium contributes to the conservation of extracellular sodium, it might appropriately be under the control of these hormones. Such a method of control could explain the impaired excretion of acid and ammonia by adrenalectomized animals, and the refusal of the kidneys to part with sodium and excrete alkaline urine during severe deficiencies of sodium. The gradually increasing output of ammonia which may occur during the first few days of a prolonged acidosis could also be explained by an enhanced secretion of adrenal cortical hormones in response to the stress of progressive depletion of the body's stores of fixed base. The difficulty about attributing a rather specific process of adjustment to adrenal hormones is that almost everything which can happen to the organism may constitute a "stress" which is supposed to increase the output of adrenal cortical hormones.

VI. THE REGULATION OF RENAL FUNCTION.

There is something to be said for the somewhat unorthodox classification of factors which control the function of the kidneys shown in the accompanying list

1. INTRINSIC FACTORS These may be either
   - i) Limitations imposed by, or
   - ii) Properties depending upon,
     the manner in which the kidneys operate.
2. EXTRINSIC FACTORS These may be either
   - i) "Inevitable,"
   - ii) Nervous, or
   - iii) Hormonal

The extrinsic nervous and hormonal factors are too closely interdependent to be discussed separately. Their co-operation to provide adjustments of renal function to meet the changing needs of the body will be discussed in the next chapter upon the regulation of the volume of the body fluids "Inevitable" responses of the kidneys to extrinsic factors (2,i) may be quickly dismissed here as almost trivial physiologically, although they are sometimes important under pathological conditions The anuria which occurs in shock when the systemic blood pressure falls precipitously and deprives the kidneys of a circulation is an inevitable response to factors outside the kidney. Although the conservation of water during shock is important to the organism, this anuria is not an adaptation of renal function to prevent the loss of water from the body, but a direct consequence of the fact that the kidneys cannot operate without blood. The remainder of the present chapter will be

devoted to the regulation of renal function by some intrinsic factors.

Two examples may be given of responses determined by limitations imposed by the manner in which the kidneys operate (1,1).

A. Osmotic diuresis during dehydration. The profuse diuresis which occurs even in dehydrated men and animals, when 10% sodium chloride is administered to them intravenously, and during which the urine becomes less concentrated than it was previously, has already been discussed because of the light which it throws upon the manner in which the kidneys excrete concentrated urine. Although in the course of this diuresis the administered salt is eliminated rapidly if incompletely from the body, the diuresis is in no true sense a renal response directed to the purpose of eliminating the salt. Water is lost at the same time, out of proportion to the amount of salt eliminated, and the already abnormally high osmotic pressure of the body fluids is increased still further by the action of the kidneys. It must be added in fairness to the kidneys that the increase in osmotic pressure of the extracellular fluid is not wholly evil, for it causes a movement of water from the cells to the extracellular fluids and blood which is of value in sustaining the circulation during dehydration from lack of water. Moreover the correction of dehydration is not primarily the task of the kidneys. The kidneys can to a limited extent conserve water which is present in the body, but they cannot generate it when it is lacking; there is another mechanism, that of thirst, operated by rising osmotic pressure in the body fluids, which, if it can be satisfied, leads to the replacement of body water.

B. The changes in renal function which occur when sodium is so seriously depleted that the body fluids become hypotonic provide another example of adverse conditions which are beyond the capacity of the kidneys to compensate. The osmotic pressure of the body fluids cannot be greatly lowered in a

normal person by drinking water, because the kidneys can excrete water as fast as the intestinal tract absorbs it, but hypotonicity can be induced experimentally by two methods In animals this has been done by introducing isotonic solutions of glucose into the peritoneal cavity, and withdrawing them a few hours later after they have come into equilibrium with the sodium salts of the extracellular fluid; sodium salts are thereby removed from the body but water is not. The loss of its major osmotically active solutes lowers the osmotic pressure of the extracellular fluid, and water moves into the cells. The skin and eyeballs of animals treated in this way lose their turgor, and the peripheral circulation may fail from the reduction in circulating blood volume McCance produced a similar deficiency of sodium in human subjects and maintained it for many days by the combination of a sodium-free diet with forced sweating in a heat-bath and the drinking of distilled water.[80] In addition to the effects described in animals, there were a number of interesting alterations in renal function. During the first three or four days of progressive depletion the body weight fell as the kidneys excreted water in proportion to the sodium chloride which was removed in the sweat, and during this time the tendency for loss of sodium to lower the osmotic pressure of the body fluids was successfully compensated. After the first few days, although sodium was still being removed, the weight of the subjects ceased to fall; instead it began to fluctuate rather irregularly. At this time, the tonicity of the extracellular fluids, as indicated by the concentration of chloride in the plasma, fell by about 20%. The kidneys seemed to have given up the struggle to maintain the osmotic pressure of the body fluids with a diminishing amount of extracellular solute; the conflict between regulation of total concentration and regulation of volume had been solved for a few days by sacrificing volume to tonicity, but now tonicity seemed to be sacrificed to volume. During this later phase renal function became abnormal in a

number of ways: 1) There was a considerable reduction in glomerular filtration rate. 2) The excretion of sodium in the urine, which was minimal, indicating maximal renal conservation of sodium, did not increase during respiratory alkalosis induced by voluntary over-breathing. The kidneys excreted no sodium, there was no diuresis, and the urine could not be made alkaline. 3) Despite the low osmotic pressure of the extracellular fluids, water diuresis was impaired. One subject drank water with the intention of obtaining a large minute volume to facilitate tests of renal function during the day; but no diuresis followed — until the middle of the night. Two factors may have contributed to this impairment of the diuretic response to ingested water; less sodium might have been left over from proximal reabsorption to bring water into the distal tubule to be released as "osmotically free water"; and in addition water was probably not excreted normally in the urine because it had left the blood by another route — the low extracellular osmotic pressure had allowed it to disappear into the cells so that it was not presented to the kidneys to be excreted.

These disturbances of renal function suggest that when the kidneys ceased to guard the tonicity of the body fluids but appeared still to be conserving their volume, they did so because of an inherent limitation brought out by the stress of circumstances, rather than as an adaptive response. The volume of the extracellular fluid was not maintained; water was conserved, but only in the cells, and because the kidneys had failed to maintain extracellular tonicity. The kidneys did what they could by preventing loss of sodium in the urine, but they did not raise the osmotic pressure of the body fluids by excreting water. This may have been because water was kept from them, and once again it must be added in fairness to the kidneys that they can no more manufacture sodium to correct hypotonicity than they can generate water to counter the effects of dehydration. These limiting factors are to a certain extent extrinsic, for the kidneys

can only regulate the osmotic pressure of the extracellular fluids if the diet provides an adequate intake of water and sodium salts. Renal regulation is by disposal of surpluses.

The excretion of urea is regulated automatically in a manner which is a direct consequence of the mechanism of excretion. (1,*ii*). It will be recalled that when the minute volume is 2 ml. or more, the clearance of urea is about two-thirds of the rate of glomerular filtration, but that the clearance falls somewhat at lower minute volumes because of passive diffusion of urea from the tubules back to the blood. The rate of excretion of urea depends chiefly upon the amount filtered, and upon the minute volume to a smaller extent. Hence if the glomerular filtration rate remains constant the rate of excretion is, apart from small variations with minute volume, directly proportional to the concentration of urea in the plasma. (The rate of filtration is $F.P_u$, where F is the filtration rate of plasma and P_u is the concentration of urea in the plasma.) Three examples of adjustment of the excretion of urea to meet altered circumstances *may* now be considered.

1. During periods of economy of water, when the minute volume is low and back-diffusion is increased, the decreased rate of excretion must increase the concentration of urea in the plasma if the body continues to produce it at a constant rate. This raises the rate of filtration of urea, and compensates for the increased passive reabsorption, an adjustment which can be achieved by alterations in blood urea within the normal limits.

2. If the rate at which urea is produced in the body increases, as by an increased breakdown of protein, a moderate increase in the blood urea concentration will allow excretion to keep pace [illegible]

3 [illegible] iminished, by [illegible] plasma is reduced, perhaps drastically. If however the blood urea concentration is increased in proportion to the diminution in

glomerular filtration rate, the filtration rate of urea, *FP*, will remain as large as before; the same amount of urea as formerly will now be contained in the smaller volume of glomerular filtrate, and excretion will keep pace with production. A high, but constant, level of blood urea does not imply that the excretion of urea is still lagging behind its production, and it implies nitrogen retention only in the sense that there is an abnormally large amount of urea in the body; this is the counterpart of the high concentration in the blood which enables the kidneys to eliminate urea as fast as it is formed. A rising concentration of urea in the plasma implies nitrogen retention in the more sinister sense that urea is accumulating in the body, and that excretion is not keeping pace with production. It is not always clear when we speak of "nitrogen retention" whether we mean that urea is accumulating in the body, or that urea has accumulated in the past, but a steady state has now been reached; the distinction is not merely academic, for one implies progressive and the other stationary disease.

The fact that a higher concentration in the blood enables the rate of excretion of urea to keep up with its production is no more evidence of an adaptive response of the kidney to get rid of urea more quickly, than the production of a faster flow of water through a pipe by a greater head of hydrostatic pressure is evidence of an adaptive response on the part of the pipe. It is a property of pipes that liquids flow through them more quickly under greater heads of pressure; and it is a property of kidneys that they excrete every substance which has a constant clearance at a rate proportional to its concentration in the plasma. A dam filling at a constant rate and emptied through a sluice will serve as a simple model of the relations governing the excretion of urea. So long as the rate of outflow through the sluice is equal to the inflow, the depth of the water will remain constant. If the cross-section of the sluice is reduced to one half, the rate of outflow will fall to one half of the initial rate, and will become

less than the inflow. This discrepancy will persist, though diminishing, until the depth of water has been doubled, when outflow will again equal inflow at the original rate, and the extra head of pressure obtained by doubling the depth will compensate for the greater resistance to flow through the narrower opening. This compensatory adjustment to maintain the rate of emptying is a simple property inherent ...

Pl...

ur...

wh... functioning nephrons has been reduced, the rate of solute excretion *per nephron* is abnormally high. [70] An unusually high rate of solute excretion per nephron is encountered in normal kidneys, with an undiminished number of nephrons, during osmotic diuresis. It has already been pointed out that the urine can never be much concentrated when large volumes are formed, and during extreme osmotic diuresis it may become almost isotonic with the plasma. Ordinary minute volumes in a patient with diseased kidneys therefore correspond to high rates of osmotic diuresis in a normal person, and the patient's kidneys are compelled to work permanently under the conditions of osmotic diuresis. Consequently no single urinary solute is greatly concentrated, and this, in conjunction with the elevation of its concentration in the plasma, minimizes the back-diffusion of urea. Moreover the urine as a whole cannot be much concentrated, which may be the explanation of the "Fixed specific gravity of 1010" (indicating approximate isotonicity with the plasma), and the associated increase in the daily urinary output, of patients with advanced renal disease. The power to concentrate the urine is among the first renal functions to be impaired by destructive disease or by experimental partial nephrectomy, because the maximal concentrating power of normal kidneys is only exhibited when the amount of solutes excreted per minute is small enough to be dissolved in less than 1 ml. of concentrated urine. The solutes normally

excreted in it determine the volume of the urine in the same manner as extraneous osmotic diuretics; and only a little more than the basal rate of solute output is needed to produce conditions in which conservation of water is restricted by a maximal transfer rate before the osmotic ceiling is reached. Hence when the number of nephrons is reduced, the surviving ones soon become few enough to be overloaded by a normal daily output of urinary solutes, for it is the solute output per nephron *that is* important, and the body continues to produce urea at an undiminished rate whilst the number of nephrons available to excrete it is dwindling.

VII. THE REGULATION OF THE VOLUME OF THE BODY FLUIDS.

The total amount of water *in the body* depends upon a dynamic balance between gains and losses. Gains arise principally from intake in food and drink but include also water formed by oxidation of the hydrogen of metabolites. Some of the losses cannot be avoided, because they are inseparably linked with essential physiological functions. The most important ways in which the body loses water unavoidably may be summarised in the form of a list

1. Insensible perspiration, which is practically invariable. It consists of the slow evaporation of about 400 ml of water per day from the moist surface of the skin between the sweat ducts.
2. Loss in expired air. Whatever the relative humidity of the inspired air the expired air is saturated with water vapour at the internal temperature of the lungs This results in a loss of water which is not large (about 400 ml. per day) under ordinary conditions, but may reach a crippling magnitude at extremely high altitudes where the inspired air is dry, and where the ventilation rate requires to be enormously increased.
3. Loss in sweat. This varies according to climatic conditions and activity from negligible amounts to a daily loss of about half the volume of the extracellular fluids and is dictated by the requirements of temperature regulation
4. Loss in faeces. This is unimportant under normal conditions, but becomes important in severe diarrhoea.
5. Renal loss of the least amount of water required to excrete

the urinary solutes in maximally concentrated urine. This is determined by the amount of solutes to be excreted.

Intake and metabolic production of water must at least equal the sum of the unavoidable losses, and may exceed it, in which case the kidneys excrete the excess water in a more dilute urine.

The mechanism which normally ensures that the intake of water keeps pace with its loss by all routes is thirst, and experiments in which the distribution of water in the body has been altered without changing its total amount have indicated that the stimulus which gives rise to the sensation of thirst is cellular dehydration. Thus animals in which the osmotic pressure of the body fluids had been reduced by peritoneal dialysis with isotonic solutions of glucose showed, as has already been mentioned, signs and symptoms of severe dehydration, but they were not thirsty. The severely sodium-depleted human subjects of McCance's experiments were not thirsty although they had lost large amounts of body water as well as sodium chloride. In both cases the osmotic pressure of the extracellular fluids was low, and the cells were presumably overhydrated. Conversely, the intravenous injection of quite small amounts of hypertonic saline is a sure method of evoking thirst. Such solutions are also effective if they are injected into the internal carotid artery, so that thirst may be signalled not by the dehydration of cells in general so much as by the dehydration of especially sensitive cells situated in the territory supplied by the internal carotid artery. The response to thirst is moreover sometimes so rapid and so precise as to suggest that the central nervous system participates in it.[36] Thirsty rats may take in a single draught the amount of water which will, when it has been absorbed and distributed some time after they have stopped drinking, restore the normal hydration of their bodies. There seem to be receptors somewhere which can signal not only that the animal needs water, but how much it needs, and these might be the same *osmoreceptors* which signal the neurohypophysis to release its

antidiuretic hormone. It must be admitted however that thirst is not always so well adjusted to the quantitative need for water. Some dogs satisfy their thirst in one draught, and others in a number of smaller ones; and those which take a single draught may ingest a volume of water which is characteristic of the individual dog rather than appropriate to its need for water.[40] Drinking in man is controlled by the higher parts of the nervous system in a manner which may bear little relation to the need for water.

The amount of water in the body depends upon the amount of protoplasm, for this has a fairly uniform proportion of water associated with it in the cells, although the proportion depends to some extent upon the age of the animal and upon its metabolic activity. The intake of food plays a minor part in regulating the total amount of water in the body in so far as it affects the mass of protoplasm. Other factors, like the growth hormone of the anterior pituitary, and insulin, play similar minor roles, but the variations in total body water which they produce are long-term ones Short-term variations, with which this chapter is concerned, are compensated mainly by the kidneys The problem of how the water balance of the body is maintained from hour to hour therefore resolves itself into the problem of how the kidneys control the losses of water and of electrolytes; and the more intricate problem of how the body, provided with kidneys whose function can be regulated within rather wide limits, uses these to adjust the volume as well as the composition of its fluids. This control which the body exerts over the activity of the kidneys is mediated partly by nerves and partly by hormones.

Direct nervous control of renal function is generally held to be relatively unimportant. The rich sympathetic nerve supply which the kidneys possess is supposed to influence mainly their blood vessels, and those experimenters who have been most careful to avoid the complicating effects of trauma and of

anaesthetics have usually been least successful in demonstrating a nervous control of renal function. But some recent suggestions that sympathetic nerves distributed to the tubular epithelial cells control the reabsorption of sodium will be referred to later.

There seems no doubt that tubular function, in so far as it is not autonomous, is controlled predominantly by circulating hormones, and that these provide the kidneys with efferent pathways which correspond to the motor nerves of voluntary muscles or the secreto-motor nerves of other glands. But these hormones provide only the efferent side of the control; and the afferent limbs of reflex arcs which employ the kidneys as their effector organs are probably nervous to a greater extent than has been realised. There is evidence, moreover, that afferent paths converge from a wide field upon the humoral final common path to the kidneys. Afferent impulses travelling in nervous pathways are known to play a part in the renal response to alterations in the tonicity of the body fluids, and afferent impulses arriving in the nervous system along other pathways may interfere with the normal responses to altered tonicity. Moreover nervous paths must be employed to signal changes in volume, since these can occur without any alteration in composition.

ENDOCRINE FACTORS

The endocrine glands which influence the kidneys will first be listed and then the factors which govern the release of their hormones will be considered.

1. The neurohypophysis. This gland releases an antidiuretic hormone which increases the reabsorption of water by the renal tubules, causing a fall in minute volume typically accompanied by an approximately proportionate increase in the concentration of the urine.
2. The adenohypophysis. The anterior lobe of the pituitary

gland plays a part in the maintenance of the kidneys and of their function which is not fully understood. There may be a specific renotrophic hormone, or the effects sometimes attributed to it may be those of the hormones called out by the thyrotrophic, adrenocorticotrophic and gonadotrophic hormones. In so far as permanent diabetes insipidus cannot be induced experimentally by total hypophysectomy, but requires a functioning adenohypophysis, the renal actions of the anterior and posterior lobes of the pituitary are partly antagonistic and partly synergistic.

3. The thyroid. Possibly through its general effect of stimulating metabolism the thyroid has some influence upon the size of the kidneys and also upon the intestinal absorption and the renal excretion of water
4. The suprarenal cortex Certain steroids secreted by the suprarenal cortex have two major effects upon renal function.
   i) They promote the tubular reabsorption of sodium. In Addison's disease the excretion of sodium continues although its concentration in the serum is subnormal, a defect of function which can be corrected by adrenal cortical extracts or by synthetic steroids such as deoxycortone.
   ii) They promote water diuresis. *They might do this by* promoting the distal reabsorption of sodium which serves to release "osmotically free water", or by a more specific antagonism to the antidiuretic hormone where it controls back-diffusion of water. Adrenal cortical hormones might, for example, close the pores which the antidiuretic hormone seems to open. It seems that adrenal cortical hormones are required to provide an essential background for the regulation of the excretion of water, though they do not directly control it The failure of diabetes insipidus to be established when the

adenohypophysis is destroyed may be explained in part by lack of adrenocorticotrophic hormone and of the steroids which it mobilizes. Chalmers and Lewis have described the restoration of a normal diuretic response to water by adrenocorticotrophic hormone in patients with panhypopituitarism.[12] The diuretic response to water is impaired in patients with Addison's disease, but it may be incorrect to attribute the poor water diuresis of these patients directly to a lack of suprarenal hormones and of their effects within the kidneys, for the salt-deficient subjects of McCance's experiments showed a similar impairment of water diuresis, although the absence of sodium from their urine indicated that their output of adrenal cortical hormones was unimpaired, if not greater than normal.

5. The gonads. Testosterone has been found to stimulate the growth of the renal tubular epithelium; and progesterone shares some of the salt-retaining properties of adrenal steroids
6. The parathyroids. The parathyroid hormone increases the *renal excretion of calcium, probably by raising its concentra*tion in the plasma. This in turn may be partly due to an influence upon the reabsorption of phosphate by the renal tubules, but also to an effect upon the bones.

Two of these hormones possess actions which might be employed to regulate the volume of the body fluids. These are the antidiuretic hormone of the neurohypophysis and the adrenocorticotrophic hormone of the adenohypophysis. The antidiuretic hormone, working on a background of basal adrenal cortical function, could be used to signal the kidneys to retain water, and so to adjust the volume of water in the extracellular compartments to the amount of sodium in them. This would both stabilize the extracellular osmotic pressure and regulate the volume of water in the cells by controlling the distribution

of water between the extracellular and intracellular compartments The adrenocorticotrophic hormone working upon a background of constant extracellular osmotic pressure provided by the antidiuretic hormone, could then be used to signal the kidneys to release or retain sodium and so vary the total volume of water in the extracellular compartments. These two hypophyseal hormones look like the reins by which the body drives the paired kidneys. Between them they could be used to regulate the total quantity of water in the body and also its distribution. As might be anticipated from the manner in which these hormones are released, and from their peripheral actions, the one direct upon the kidney, and the other indirect, the control of tonicity is precise and quick-acting, that of volume less precise and usually rather slow, allowing characteristic fluctuations with an irregular periodicity of several days.

The central problem of how the volumes of the body fluids are regulated therefore shifts to that of discovering what controls the release of the two key hormones Since the sites from which these are released are the two morphological divisions of the hypophysis, the controlling mechanisms are likely to operate through the hypothalamus. The factors which govern the release of the antidiuretic hormone will be taken first, because knowledge of them is fuller and more precise, and because the fixing of osmotic pressure sets the stage for the regulation of volume.

THE RELEASE OF ANTIDIURETIC HORMONE

Far and away the most important single factor which causes the antidiuretic hormone to be released from the hypophysis is an increase in the osmotic pressure of the extracellular fluid. An increase of 1% in the osmotic pressure of the blood flowing through the internal carotid artery is sufficient to release the hormone, and as Verney has so beautifully shown, this is the basis of a quick-acting homeostatic mechanism which regulates

the osmotic pressure of the body fluids with great precision.[98, 99] When the hormone reaches the kidneys in arterial blood whose osmotic pressure is beginning to rise, the kidneys reabsorb a fluid more dilute than the plasma and dilute the venous blood which leaves them. When the osmotic pressure of the extracellular fluid falls as water is absorbed into the blood, the familiar brisk water diuresis which checks the fall in osmotic pressure within the body occurs after a characteristic delay of 10 to 15 minutes while circulating hormone disappears. The sensitive end-organs (osmoreceptors) which respond to alterations in extracellular osmotic pressure have not yet been certainly located, although they may be associated with fluid-containing vesicles, 50 to 60 μ in diameter which have been found in the supra-optic nuclei. The efferent nervous pathway to the neurohypophysis is in the supra-optico-hypophysial tract.

Nervous impulses from osmoreceptors may contribute to the regulation of tonicity in two quite distinct ways. Patients with diabetes insipidus and animals in which this condition has been imitated by dividing the supra-optico-hypophysial tract regulate the osmotic pressure of their extracellular fluids almost as satisfactorily as normal animals provided that they have unrestricted access to water. They experience thirst sooner and more urgently than normal animals, but so long as their thirst can be satisfied the tonicity of the body fluids can be kept within normal limits. The osmoreceptors cannot now signal the neurohypophysis to release its antidiuretic hormone, but the sensation of thirst must be aroused in a normal manner as soon as extracellular osmotic pressure begins to rise. Hence it appears that the osmoreceptors perform two functions; they signal thirst to the cerebral cortex, and they cause antidiuretic hormone to be released, so that the loss of water in the urine is held in check until more water has been found and ingested. The peculiar value of the antidiuretic hormone is that it enables an animal to maintain its water balance on a smaller intake and

turnover of water than would be required otherwise. It also reduces the amount by which the tonicity of the body fluids fluctuates between *different occasions* of drinking, and reduces the necessary frequency of these occasions Without it a great deal of time and energy would have to be spent in the pursuit of water, and a great deal of heat would have to be wasted in raising it to the temperature of the body. Thus a patient with diabetes insipidus who drank 20 litres of water at 17° C. each day, and excreted it at 37° C., would lose 400 Cal. in warming it — more than 10% of his total metabolic heat production

An effector pathway leading from the hypothalamus might be expected to be a final common path which could be activated by afferent impulses converging from a wide field, and in fact many factors besides alterations in osmotic pressure affect the release of antidiuretic hormone. Most of these are merely listed with little comment. A paper by Strauss and others gives useful references [82]

FACTORS AFFECTING THE RELEASE OF ANTIDIURETIC HORMONE

1. Hypnosis
2. Cold.
3. Alcohol.

These all inhibit the release of antidiuretic hormone. The inhibitory effect of alcohol at night may contribute to dehydration next morning.

4. Conditioned *reflexes may either stimulate or inhibit*
5. Local stimulation of the hypothalamus, either electrically through buried electrodes, or by the injection of small quantities of hypertonic saline, releases antidiuretic hormone. The experiments with hypertonic saline might indicate that there are osmoreceptors in the hypothalamus, but they need not do so because the solutions employed were strong enough to stimulate any nerve axons with which they happened to come in contact, and hence could have

initiated impulses at any part of the reflex arc between the osmoreceptors and the neurohypophysis.

6. The smoking of tobacco, especially by those unaccustomed to it, or the intravenous injection of about the same amount of nicotine as would be absorbed from the smoke, and injections of acetyl-choline, all lead to release of antidiuretic hormone. These drugs presumably act at synapses on pathways leading to release of the hormone.
7. Anaesthesia and sleep are accompanied by the release of the antidiuretic hormone. Its release during anaesthesia precludes a response to exogenous hormone, and early work on anaesthetized animals showed that posterior pituitary extracts possess a diuretic action, which may be secondary to their effect upon systemic blood pressure, and which caused confusion when antidiuretic effects began to be encountered in unanaesthetized animals. Release of the hormone during sleep has the convenient result that most of the daily output of a litre and a half or so of urine is excreted by day, so that the urine secreted during the night may be accommodated in the bladder, and sleep may be uninterrupted. The urine obtained when the bladder is emptied on rising is normally more concentrated than any specimen passed during the day, and is to be preferred for routine clinical examinations, since abnormal chemical constituents present in higher concentrations and are more certain to be detected, and erythrocytes remain unhaemolysed.
8. Muscular exercise is usually accompanied by release of antidiuretic hormone.
9. Fainting, pain, and unpleasant emotions lead to the release of antidiuretic hormone.
10. Alterations in the volume of the blood and of the extracellular fluids of the body, as well as alterations in their tonicity, may lead to the release of antidiuretic hormone

or may inhibit its release when this might otherwise be expected.

11. Posture influences the output of the hormone, which tends to be increased in the erect posture and inhibited for a time by recumbency. The effect of sleep over-rides the usual postural influence of recumbency in waking subjects, the colloid osmotic pressure, haemoglobin concentration and packed cell volume are usually at their lowest in the morning after a night of recumbent sleep because the haemodilution produced by the posture (which is associated in waking subjects with diuresis) is augmented by the antidiuretic effect of sleep.
12. Time of day has an influence which is probably secondary to the sequence of activities usually associated with it. Sleeping and wakefulness appear to be the most important factors causing diurnal modifications in the excretion of water.
13. The release of antidiuretic hormone may be inhibited by breathing 5 — 7% carbon dioxide.[2]

A little will be said about the effects of emotion and of conditioned reflexes before passing on to more detailed discussion of the responses of the kidneys to alterations in the volume of the blood and of the extracellular fluids.

EMOTION

A number of responses to emotional disturbances in man and to upsetting circumstances in animals have been recognized. Verney has described how a nocuous stimulus (an electric shock or a nasty noise) may check water diuresis in dogs in a manner which can be matched by the injection of a suitable small dose of posterior pituitary extract. This inhibition of water diuresis through the release of antidiuretic hormone does not occur in all dogs; some secrete adrenaline, which produces a shorter, sharper and less complete antidiuresis of its own, but prevents

the release of antidiuretic hormone. After denervation of the *suprarenals, which prevents the secretion of adrenaline, however*, dogs which did not previously release antidiuretic hormone on receiving the nocuous stimulus do so. Since it has been shown that adrenaline does not inhibit the action of the antidiuretic hormone within the kidney, the difference in response between different dogs seems to depend upon whether they secrete antidiuretic hormone before adrenaline or not. Antidiuretic responses typical of the effects of the antidiuretic hormone have been encountered in well-hydrated patients or experimental subjects who have been hurt, or anxious, or have become alarmed about the procedures to which they were being subjected.

A different kind of response, diuretic instead of antidiuretic, has been described in patients, especially hypertensive patients, in response to catheterization, or the anticipation of this procedure, or to the appearance before them of a gowned surgeon who discussed their case. [58] This was in female patients who had been deprived of water beforehand with the object of reducing the minute volume of urine, and it was proved to be an osmotic *diuresis secondary to diminished tubular reabsorption of sodium* chloride. Since it was not inhibited by exogenous pitressin, it does not imply that the more usual increase in output of antidiuretic hormone with emotional stress had not occurred; the conditions were unsuitable for an increased output to be demonstrated. One of these patients also produced a profuse water diuresis after cystoscopy although she had been deprived of water for 24 hours beforehand. The output of urine rose to 18 ml. per minute, and the specific gravity was in the range from 1002 to 1005. This large diuresis can only be ascribed to a suppression of the secretion of antidiuretic hormone under *conditions of dehydration* [59]

It thus appears that emotional stimuli may modify renal function in ways which appear contradictory at first sight, but

are not so because they depend upon the conditions under which the observations are made. These emotional responses are of practical importance because they may lead to fictitious results in procedures which are employed for diagnostic testing. Thus either a water diuresis or an osmotic diuresis occurring during a concentration test could lead to erroneous diagnosis of renal insufficiency, and emotional antidiuresis during a dilution or a water excretion test would be equally confusing.

CONDITIONED REFLEXES

Both diuretic and antidiuretic responses may be conditioned Bykow found that when water diuresis had been repeatedly produced in dogs by rectal administration of water, it was sufficient to make the customary preparations without injecting any water. Diuresis would occur as though the usual dose of water had been given An organ tone sounded when water was administered could be made into a conditioned stimulus, and differentiated from other tones unassociated with the administration of water. Even the experimental room might become a conditioned stimulus, so that the dogs gave diuretic responses in one room and antidiuretic responses in another Antidiuresis has also been described in response to the sound of the induction coil used for administering electric shocks.

These responses to emotion and to conditioned reflexes indicate that neurones originating in the cerebral cortex impinge, probably in the hypothalamus, upon reflex arcs which modify the output of antidiuretic hormone in response to alterations in osmotic pressure They also have a bearing on reproducibility as a criterion of the significance of experimental results. They suggest that reproducibility in a series of animals may be more significant than reproducibility in the same animal on a number of occasions, and that we should be on our guard lest the increasingly fashionable "trained unanaesthetized animal" becomes an animal trained to give the right answer

The safest kind of experiment is that in which different responses are recorded under standardized conditions.

The list on page 133 is one mainly of disturbing factors. An increase in the osmotic pressure of the extracellular fluids remains the most important single stimulus which brings about the release of the antidiuretic hormone. There are however important indications that the release of this hormone may be influenced reflexly by alterations in the volume as well as in the concentration of the body fluids. Reflex effects of blood volume upon the renal excretion of water and of sodium may conveniently be considered together. The discussion which follows will be largely confined to effects of the volume of blood, for Peters has suggested that the kidneys are indifferent to the volume of extracellular fluid outside the blood vascular system.[85] In general, moreover, *alterations in the total volume of extracellular fluid are likely to be associated with alterations in the volume of blood in the same direction,* — unless the liver intervenes by drastically changing the total amount of circulating plasma protein. *It is important to realise that* the amount of plasma protein in the circulation must play a major part in determining what fraction the plasma forms of the whole extracellular fluid volume, and that this is a factor which has been left out of account more often than not. *Warren et al.* have adduced evidence that even if the amount of plasma protein is seriously deficient, a large increase in the volume of extracellular fluid can prevent the blood volume from falling so much that the circulation fails.[103] *The volume of circulating* blood may therefore be used as an indicator of the volume of extracellular fluid, though as one whose sensitivity depends upon how much protein there is in the plasma.

THE EFFECT OF BLOOD VOLUME UPON RENAL FUNCTION

Clinical observations have long suggested a connection between renal function and the volume of circulating blood.

Borst pointed out that the kidneys tended to retain sodium actively when the blood volume was diminished by haemorrhage or shock, though he has probably over-simplified the problem by suggesting that the rate of excretion of sodium chloride by the kidneys is simply proportional to the cardiac output.[7]

Since extra-renal losses of water through the skin and lungs are always going on, *renal retention of sodium will raise the osmotic pressure of the extracellular fluids, which in turn will lead to* retention of water under the influence of the antidiuretic hormone, and, by stimulating thirst, to the drinking of more water. This sequence serves normally to maintain the volume of the extracellular fluids, and also of the blood if the concentration of plasma proteins remains adequate. If plasma protein is grossly deficient, however, the salt reabsorbed from the tubules and the water absorbed from the intestine will not remain in the blood stream; blood volume will not be restored, the stimulus which leads *to retention of sodium by the kidneys will continue* to act; and unless the dietary intake of salt is restricted, salt will accumulate with water in the extravascular parts of the extracellular fluid compartment as oedema fluid The reduced blood volume here leads to a continuing increase in the volume of the extracellular fluids although this is already greatly above the normal, a vicious cycle which is partly responsible for the gross oedema associated with nephrotic syndromes This cannot be the sole factor causing retention of water in nephrosis however, because water frequently seems to be retained in excess of sodium, *presumably by increased secretion of pituitary antidiuretic hormone, so that the serum sodium concentration may* be less than normal. When this occurs the low serum sodium concentration may itself contribute to the diminished excretion of sodium by the kidneys. For further details of renal handling of sodium the monograph of Black may be consulted [4]

It is perhaps worth stressing that mere retention of what is already inside the body cannot increase its amount, additional

quantities taken in with the diet must be retained to produce a positive balance. The salt and water which make up oedema fluid, like the fat of obese persons, get into the body through the mouth, and an important cause of oedema is thirst, secondary to the retention of sodium chloride. Hence the importance of salt-free diets in treatment, and the value of ion-exchange resins which abstract sodium from the intestinal contents.

Extreme reductions in the volume of circulating blood may reduce the excretion of salt and water by depriving the kidneys of blood. It was once suggested that lesser variations might produce their effect by means of parallel alterations in the rate of glomerular filtration, the rate of tubular reabsorption being constant. Such a theory was hard to prove because the day to day variations in glomerular filtration necessary to effect homeostasis would usually be too small to measure. And it soon became clear that the rate of reabsorption of sodium by the tubules was far from constant; it varied under the influence of hormones released from the suprarenal cortex by the adrenocorticotrophic hormone of the adenohypophysis.[6] Addison's disease provides a clinical example of reductions in the volume of blood and of extracellular fluid which are not associated with increased renal reabsorption of sodium; and here the adrenal cortical link is missing from the chain.

Many experiments have been performed to test the hypothesis that the excretion of water and sodium varies with the volume of circulating blood. Some of these are listed in Table 3, classified according to the effects they produced, with "antidiuretic" procedures shown on the left and "diuretic" procedures on the right. References are given to the more recent work. Information about the earlier work is given in the article by J. P. Peters which has already been quoted [65]

It has been emphasised that the excretion of water by the kidneys is not completely independent of the excretion of salt, because an increase in the amount of solute left in the tubules

reduces the amount of water which can be reabsorbed, and increases the volume of the urine Consequently the alterations in reabsorption of water which are listed in Table 3 were often in part secondary to changes in the reabsorption of salt, and it is not always possible to be certain how much of a change in water reabsorption should be attributed to an alteration in the output of antidiuretic hormone. Many procedures which reduced the excretion of sodium and water also reduced the rate of glomerular filtration and the circulation through the kidneys, but not enough to account for the observed alteration in renal function without an increase in tubular reabsorption in addition Moreover some of the gentler procedures influenced the reabsorption of sodium or of water without affecting the filtration rate or the renal circulation Thus Welt and Orloff (B,7) found that intravenous infusions of iso-oncotic (4%) solutions of salt-poor human serum albumin dissolved in normal saline, produced in reclining human subjects a typical brisk water diuresis due to suppression of the release of antidiuretic hormone, and which occurred without any discoverable change in glomerular filtration rate [104] Glomerular filtration rates were also usually unchanged by the infusion of hyperoncotic solutions of albumin.

A first glance at Table 3 suggests that the hypothesis of a connection between renal excretion of sodium and water and the volume of circulating blood has been reasonably well substantiated. The "antidiuretic" procedures A, 1 to 5 and C, 1 to 5 all reduced the volume of circulating blood, and the "diuretic" procedures B, 1, 2, 6 and 7 and D, 1, 4 and 5 all increased the volume of blood or of extracellular fluid.

The effects of the procedures A, 6 and C, 6 however point a warning to look out for other factors The intravenous infusion of about 75 grams of salt-poor human serum albumin as either a 25% or a 10% solution in 0 9% sodium chloride substantially increased the volume of circulating blood, but this was

TABLE 3

Some procedures which modify renal excretion of water and electrolytes. (References in parenthesis)

A Procedures followed by *increased reabsorption of water.*

1. Haemorrhage, shock. (7)
2. Passive erect posture. (26)
3. Inflation of pneumatic cuffs around proximal parts of limbs. (29, 107)
4. Sitting up (after lying flat) (47, 100)
5. Intraperitoneal injection of hyperoncotic albumin solutions (14)
6. Intravenous infusion of hyperoncotic albumin solutions (34, 66, 104)
7. Abdominal compression (8, 9)
8. Inflation of balloon in vena cava (27, 28)

B. Procedures followed by *decreased reabsorption of water.*

1. Plasma transfusion
2. Lying down. (54)
3. Breathing air at pressures below that of the surrounding atmosphere. (80, 81)
4. Inflation of pneumatic cuff around neck of sitting subject. (47, 100)
5. Compression of legs by elastic bandages (49)
6. Rapid infusion of isotonic saline in recumbent, hydrated subjects (45, 92)
7. Intravenous infusion of isooncotic albumin solutions (104)
8. Lowering central venous pressure by digoxin in patients with congestive cardiac failure. (23)

C. Procedures followed by *increased reabsorption of sodium*

1. Haemorrhage, shock (7)
2. Passive erect posture (26)
3. Inflation of pneumatic cuffs around proximal parts of limbs. (29, 107)
4. Sitting up (after lying flat). (47, 100)
5. Intraperitoneal injection of hyperoncotic albumin solutions. (14)
6. Intravenous infusion of hyperoncotic albumin solutions (34, 66, 104)
7. Abdominal compression. (8, 9)
8. Inflation of balloon in vena cava. (27, 28)

D Procedures followed by *decreased reabsorption of sodium.*

1. Lying down (54)
2. Inflation of pneumatic cuff around neck of sitting subject (47, 100)
3. Compression of legs by elastic bandages (49)
4. Intravenous infusion of hypotonic saline during water diuresis (93)
5. Intravenous infusion of isotonic saline.
6. Lowering central venous pressure with digoxin in patients with congestive cardiac failure (23)

followed by an antidiuretic response which would have seemed more appropriate if the volume of blood had been decreased.

The antidiuresis which occurs when the "passive erect posture" is assumed, (A & C,2) by standing quietly, leaning slightly back against a support with the muscles of the legs relaxed, or better, by tipping on a tilting table, is accompanied by a diminution in blood volume by exudation of fluid from the vessels in the lower parts of the body which leads to an increase in the concentrations of haemoglobin and of plasma proteins, and to a greater packed cell volume. Although the glomerular filtration rate is reduced, the antidiuresis is partly due to increased tubular reabsorption of sodium, and might be taken for an excellent example of enhanced reabsorption of water and sodium accompanying a diminution in the volume of the circulating blood. But if the diminution in blood volume is prevented by a slow intravenous infusion of iso-oncotic human serum albumin to offset the loss of fluid from the blood vessels, the change to the erect posture is still followed by its usual antidiuretic response. Moreover the postural increase in reabsorption of sodium and water on sitting up (A and C,4) takes place immediately, before the blood volume has had time to change Such observations indicate that the distribution of blood between various parts of the circulation may have as great an influence upon renal function as the total circulating volume. This suggestion receives further support from the antidiuretic effects of trapping blood in the periphery by inflating pneumatic cuffs round the thighs, (A and C, 3, a procedure which mimics in the recumbent subject the distribution of blood produced by quiet standing) contrasted with the diuretic effects of gathering in the blood towards the centre of the body by breathing air at a pressure below that of the surrounding atmosphere (B,3), or by compression of the legs with elastic bandages (B,5 & D,3). Some experiments have even suggested a volume receptor within the cranium, for the

reduction in excretion of salt and water which occurs on sitting up (A & C,4) may be almost wholly prevented by the inflation of a pneumatic cuff round the neck to pressures between 15 and 35 mm. of mercury. (B,4, & D,2). This procedure must increase the pressure of all the intracranial fluids as well as the volume of venous blood within the cranium, and one or other of these changes might initiate signals to the kidneys to increase the excretion of salt and water.

Adjustments to small or gradual increases in the volume of extracellular fluids without changing their tonicity are usually rather slow, and several days are required to excrete the whole of a moderate dose of isotonic saline. Massive infusions of isotonic saline, especially when they are run in quickly (doses of 3 litres have been administered to human subjects in periods of from one to two hours) may produce a rapid response if the subjects are well hydrated beforehand and are recumbent; but this rapid response is a typical water diuresis (B,6) and must be due to suppression of the output of antidiuretic hormone. The absence of a brisk water diuresis in sitting subjects demonstrates an effect of posture upon the release of the antidiuretic hormone, and the diuresis during recumbency shows that an increase in the volume of the extracellular fluids, as well as a decrease in their osmotic pressure, may suppress the secretion of antidiuretic hormone and hasten the elimination of water The more usual slow reduction in reabsorption of sodium with secondary increase in excretion of water after moderate doses of isotonic saline is presumably mediated by slow changes in adrenal cortical activity in response to alterations in the output of adrenocorticotrophic hormone. It is probably necessary to invoke another, brisker mechanism to account for the immediate change in the rate of sodium reabsorption on sitting up (C,4) This might be a direct effect of the sympathetic nervous system upon the tubules, for Kaplan and Rapoport found that division of the splanchnic nerves reduced the rate

of tubular reabsorption of sodium during osmotic diuresis in dogs [43]

When concentrated solutions of serum albumin were placed in the peritoneal cavity (A. & C,5) there was an increase in the *colloid osmotic pressure of the circulating blood*, associated with a decrease in its volume. The renal plasma flow and glomerular filtration rate were diminished, and the expected antidiuretic response, appropriate to the change in blood volume, occurred. This combination of raised colloid osmotic pressure with a reduction in the volume of circulating blood also occurs during quiet standing, and when pneumatic cuffs are inflated round the thighs. Since any reduction in blood volume caused by a loss of protein-free fluid from the vessels must be accompanied by an increase in colloid osmotic pressure, it has been suggested that a receptor sensitive to colloid osmotic pressure might be employed to signal alterations in blood volume. The paradoxical antidiuretic response to expansion of the blood volume by infusing concentrated solutions of albumin (A, C,6) would be explained if such a receptor was misled by the high colloid osmotic pressure of the infusion into signalling a decrease in blood volume when this had actually increased. But although the colloid osmotic pressure of the plasma also increases during "passive standing", the renal response to the change in posture may begin before either the volume or the colloid osmotic pressure of the blood has had time to change. The greater hydrostatic pressure in the veins and capillaries of the legs leads to exudation of fluid and sets up a compensating gradient of colloid osmotic pressure which holds further exudation in check. The increase in oncotic pressure is thus part of an extra-renal process of readjustment to the new posture, and is independent of *any direct part which oncotic pressure might play* in the reflexes which alter renal function to combat the diminishing *volume of blood. So far no localised "oncoreceptor"* has been demonstrated, although the volume of the whole vascular

and from the carotid sinus and the aortic arch. There might also be afferents from blood vessels all over the body, signalling continuously the amount and distribution of peripheral vasoconstrictor tone back to the centre. It is hard to see how the adjustments to changes in orientation with respect to gravity could be made without the information provided by such afferents. Alternatively the efferent paths to the blood vessels might branch, and convey to some subcortical centre "for information" a copy of the orders going out as constrictor impulses to the blood vessels "for action".

It seems probable that all the afferent information from the vascular system is co-ordinated and integrated somewhere, perhaps in the hypothalamus; and it is possible that besides the main vasomotor efferent pathways to the heart and the blood vessels, there is another output of nerve impulses to neurones which control the secretion of the two key hormones, and perhaps also via the sympathetic nerves to the tubular epithelium. *Another possibility is that the release of the hormones is controlled* not so much by the overall picture of vasomotor activity as by impulses arriving along a few selected afferent pathways. The latter is perhaps the likelier hypothesis, although the two are complementary rather than mutually exclusive, since there is no doubt that cortical events as well as peripheral changes influence renal function. The problem is to find suitable afferent paths for this special task.

The veins seem unable to provide suitable afferents for the whole task of evoking reflex changes in renal function to regulate the volume of circulating blood, and there are reasons for preferring afferents from the high-pressure side of the circulation. The primary function of the vasomotor reflexes is the control of blood pressure, and afferent paths from the arterial tree would seem better able than afferents from the veins to provide information of the degree of vasoconstriction required from moment to moment for this purpose. There is a factor

which has not so far been mentioned which is common to conditions associated with a diminished blood volume and those associated with central venous congestion A low blood volume leaves the arterial tree relatively empty, and constrictor tone must be increased to maintain the blood pressure. Even when there is a normal volume of blood in the body, procedures which trap blood in the periphery, such as "passive standing" or the inflation of cuffs round the thighs, and procedures which dam it up in the great veins, all reduce the volume in the arterial tree. When a balloon is inflated in the vena cava, blood is dammed up on the venous side of the circulation, with a corresponding reduction in the volume in the arteries, and their capacity must be reduced by a heightening of constrictor tone if the blood pressure is not to fall. In congestive heart failure the blood tends to be shifted over from the arterial to the venous side of the circulation, whether because a greater venous pressure is required to sustain the output of a failing heart, or because blood which the heart cannot pump into the arteries piles up in the veins. In all these conditions a reduction in the volume of the arterial tree and a higher level of constrictor tone are associated with retention of sodium and water by the renal tubules. Hence afferent end-organs in some vascular territory on the arterial side of the circulation might provide the missing pieces to enable the other pieces of the jigsaw puzzle (assembled in Table 3) to be put in their places. Two apparent exceptions, the diuretic response to breathing at reduced pressure (B,3) and that to the reduction of venous pressure with digoxin (B,8; D,6), might be due to an increased cardiac output requiring a relaxation of vasoconstrictor tone to prevent the systemic blood pressure from rising

Two sources of afferent impulses which might serve to couple renal function with the volume of circulating blood have already been discovered, but their discoverers were interested in the regulation of the circulation, and renal respon-

ses to the impulses arising from these receptors have not so far been sought for or reported. In 1935 Gammon and Bronk investigated bursts of impulses which ascended the splanchnic nerve in cats in time with the cardiac systole, and traced them to Pacinian corpuscles lying close to arteries in the root of the mesentery. [38] The frequency of the impulses which they sent out varied with the volume of blood in the mesenteric vessels. In 1937 Heymans, Bouckaert and Wierzuchowski described vasomotor reflexes, with reflex arcs passing through the medulla, from afferent nerve endings in the mesenteric vascular territory and the abdominal aorta in dogs. [39] An increase in the blood pressure in this area produced reflex vasodilation in the spleen, kidney and hind limb connected to their owner by nerves only and perfused with blood from another dog.

End-organs such as these are strategically placed to signal the state of filling of the arterial tree, for the frequency of the impulses which they send out should vary inversely with the prevailing level of vasoconstrictor activity. If the blood volume were depleted, or if blood were redistributed away from the arteries, the frequency of their afferent impulses should fall. If the impulses arriving in the hypothalamus from such areas as these inhibited the output of the key antidiuretic and adrenocorticotrophic hormones, this would go some way towards explaining not only the adjustment of renal function to regulate the volume of the blood and of the extracellular fluids, and the results of most of the procedures listed in Table 3, but also the genesis of cardiac oedema.

VIII. EPILOGUE.

The legacy of unanswered questions left like a trail along its course may be gathered together to recapitulate the main points of the foregoing description of renal function The separation of the glomerular filtrate into the reabsorbed fluid and the urine depends upon a series of unexplained processes of cellular transport, and upon equally mysterious restrictions which parts of the nephron seem able to impose upon some of the secondary passive movements which would be expected to accompany these active transfers

Some organic compounds are transported across the tubular epithelium by metabolic processes which may involve acetyl-coenzyme A, but the details of these processes are quite unknown, and in any event the active excretion of foreign substances is only an occasional exercise for the epithelial cells The relative impermeability of the tubules to most organic compounds, even to the highly soluble and non-polar urea, with its small molecular volume and ready diffusibility into cells inside the body, is no less mysterious, and of greater physiological importance. Unless the epithelial cells lining the tubules were unique in their permeability many molecules of waste products excreted in the glomerular filtrate would be passively reabsorbed by back-diffusion and would never reach the urine

Active reabsorption of sodium appears to be the mainspring which drives many of the kidney's operations upon water and electrolytes, but it is not known how sodium is transported through cell membranes against concentration gradients The associated movement of other substances is unrestricted in the proximal parts of the nephron, where reabsorption of sodium

together with anions and water can account for the obligatory reabsorption of five-sixths of the volume of the glomerular filtrate without changing the osmotic pressure or the pH of the fluid which remains in the lumen of the tubule. Anions pass freely across the epithelium of most of the nephron and preserve electrical neutrality when cations are reabsorbed. But the active reabsorption of sodium through a segment of distal tubular epithelium which prevents chloride from following in its wake, could cause hydrogen ions or other cations to move out of the cells into the lumen in exchange for the reabsorbed sodium; this would acidify the urine, and it could also account for the secretion of ammonia, and sometimes of potassium.

The cells lining another segment of the distal tubule must be able to reabsorb sodium, and to allow anions to accompany it, but to prevent the escape of water to equalize osmotic pressure. Although this water-proofing of an epithelium is exceptional in the nephron, it is found throughout the lower urinary tract, for dilute urine formed in the distal tubule stays dilute in the renal pelvis, the ureter and the bladder. But the water-proofing of the lower urinary tract differs from that of a special segment of the nephron, in being the property of a transitional epithelium, not of a single layer of cells, and in being permanent; whereas the impermeability which restricts diffusion of water from the distal tubule to the blood when dilute urine is being formed is reversibly abolished by the hypophyseal antidiuretic hormone. Whilst the nature of this facultative impermeability is unexplained, the effect of the antidiuretic hormone upon it is analogous to the action of posterior pituitary extracts upon frog skin.

The urine may be more concentrated as well as more dilute than the plasma; there must therefore be a further segment of epithelium, possibly a short one, whose cells can actively reabsorb water as such against osmotic gradients, whilst hindering the backdiffusion of all solutes from concentrated

tubular fluid to plasma. Even if smaller osmotic gradients produced by transporting water through cells are amplified by a countercurrent diffusion system to give a larger total difference in osmotic pressure between the urine and the blood, active transport of water without accompanying solutes is still an essential primary process which has yet to be explained

There seems to be a necessary minimum of processes which can transport substances actively or restrict their passive movement; transport of some organic compounds, of sodium and of water; restrictions upon the diffusion of organic compounds, and sometimes of all solutes which would move with water, of anions which would follow cations, or of water which would accompany cations and anions And even if the back-diffusion of water which the antidiuretic hormone controls is through pores in an intercellular cement, the remainder of the group must depend upon properties of cells, and not merely of non-living structures, for the gross renal functions which are the outward sign of these invisible cellular activities are abolished by metabolic poisons, by anoxia or by respiratory inhibitors. Restrictions upon spontaneous diffusion come within the terms of the modern definition of active transport as that which results in movements different in direction or in velocity from those which known gradients of electrical and chemical potential could produce. The energy which has to be expended in the course of all these operations is derived ultimately from respiration, apparently through the mediation of phosphoric esters containing "high-energy" bonds But that is just about all that is known about them

Although, therefore, the working of the kidneys can be analysed and described in terms of active transport of organic metabolites, of ions and of water, it cannot be fully explained until it is known how cells transport these substances. This problem is of interest to a wider circle of physiologists than those whose primary concern is with the kidney, for secretion

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